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(54) **GENE CAPABLE OF INCREASING PROTEIN CONTENT IN SEED, AND METHOD FOR UTILIZATION THEREOF**

(57) According to the present invention, a gene having a novel function that can cause an increase or decrease in seed protein content is searched for. A chimeric protein obtained by fusing a transcription factor consisting of a protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to

76 and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor or a transcription factor consisting of a protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 77 to 84 is expressed in a plant.

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Description

Background Art

[0001] In order to change the amount of protein in seeds, the following have been conventionally used: (1) an improved cultivation method; (2) a method for processing harvested seeds, and particularly grains such as rice grains, with an acid or bacterium; (3) molecular breeding using markers; (4) mutant screening; (5) gene recombination; and other methods.

[0002] Problems relating to the above methods and the object achieved by the present invention are described below.

[0003] According to the method (1) above, it is possible to change the protein amount, although it is only possible to increase or decrease the amount to a slight extent. In addition, although the method (2) above is effective to a certain extent for reducing the protein amount, processing of harvested seeds is labor- and time-consuming. Further, advantageous results such as an increase in protein amount cannot be obtained according to the method (2) above. According to the method (3) above, the protein amount is determined to be a quantitative trait. In order to modify such trait by a conventional breeding method, it is necessary to identify a plurality of gene loci that contribute highly to trait expression by QTL analysis, to specify the causative gene at each gene locus, and to introduce each causative gene into a desired variety by crossing. Therefore, the method (3) above is also labor- and time-consuming. With the method (4) above, a low-glutelin rice line such as LGC-1 is bred. However, the amount of remaining glutelin accounts for 30% to 50% of that in the original variety. In addition, there are problematic points common to low-glutelin rice lines. In fact, the amount of glutelin, which is an easily digestible protein, decreases to significantly below the level found in the original variety. However, this in turn causes a significant increase in the amount of prolamin, which is an indigestible protein. Therefore, the method (4) above cannot be evaluated as a method for reducing total seed protein content. In the case of the method (5) above, it has been reported that the total expression level of the prolamin multigene group was remarkably reduced, resulting in reduction of the protein content in rice seeds (Patent Document 1: WO2004/056993). However, in this case, the decrease in the total protein content is 15% at maximum, although the amount of prolamin itself decreases to 50% or less of the original amount. In addition, regarding the method (5) above, it has been reported that transcription factors specified by AT1G04550, AT1G66390, AT5G13330, and At2g30420 were overexpressed in *Arabidopsis thaliana* seeds, which resulted in, respectively, 25%, 14%, 39%, and 17% increases in protein content. Also, it has been reported that overexpression of a transcription factor specified by At2g47460 resulted in a decrease in the seed storage protein content of 13% (Patent Document 2: WO 01/35727).

[0004] In spite of the development of the above molecular breeding methods for the improvement of a variety of traits, there are still no practically available techniques to increase or decrease seed protein content.

[0005] As reasons for the above, it is considered that truly excellent genes remain undiscovered, and that new recombinant varieties that have been confirmed to have desirable effects in the test phase cannot exhibit expected effects upon practical use in different environments. In addition, a number of genes are involved in the expression of quantitative traits such as seed protein content in different steps in the control system, the metabolizing system, and other systems. Thus, it has been difficult to discover or develop truly excellent genes capable of improving quantitative traits. In order to solve such problems, an object of the present invention is to find a novel gene exhibiting remarkably high effects. Another object of the present invention is to develop a gene capable of exerting effects in a practical environment to an extent comparable to the effects exerted in the test phase.

Citation List

Patent Literature

[0006]

Patent Document 1: WO2004/056993

Patent Document 2: WO 01/35727

Summary of Invention

Technical Problem

[0007] In view of the above circumstances, an object of the present invention is to provide a technique for searching for a gene having a novel function that can cause an increase or decrease in seed protein content so as to improve such feature of a plant.

Solution to Problem

[0008] As a result of intensive studies to achieve the above objects, the present inventors found that it is possible to improve various quantitative traits and particularly to increase or decrease seed protein content via induction of expression of a chimeric protein obtained by fusing a particular transcription factor and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor (hereinafter sometimes referred to as a "repressor domain"), introduction of a particular gene encoding a particular transcription factor, or modification of an expression control region of an endogenous gene corresponding to the gene. This has led to the completion of the present invention.

[0009] The plant of the present invention is obtained by inducing expression of a chimeric protein in a plant, such chimeric protein obtained by fusing a transcription factor consisting of any one of the following proteins (a) to (c) and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor, introducing a gene encoding a transcription factor consisting of any one of the following proteins (d) to (f) into a plant, or modifying an expression control region of an endogenous gene corresponding to the gene in a plant.

[0010]

(a) A protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76

(b) A protein having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76.

(c) A protein having transactivation activity encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence shown in any of the odd-numbered SEQ ID NOS: 1 to 76.

(d) A protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 77 to 84.

(e) A protein having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in any of the even-numbered SEQ ID NOS: 77 to 84.

(f) A protein having transactivation activity encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence shown in any of the odd-numbered SEQ ID NOS: 77 to 84.

[0011] Preferably, the fusion of a functional peptide with a predetermined transcription factor causes repression of transcriptional regulatory activity, and particularly, transactivation activity, of the transcription factor in the plant of the present invention. Examples of the above functional peptide used herein include peptides expressed by the following formulae (1) to (8).

[0012]

(1) X1-Leu-Asp-Leu-X2-Leu-X3

(where X1 denotes a set of 0 to 10 amino acid residues, X2 denotes Asn or Glu, and X3 denotes a set of at least 6 amino acid residues.)

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3

(where Y1 denotes a set of 0 to 10 amino acid residues, Y2 denotes Phe or Ile, and Y3 denotes a set of at least 6 amino acid residues.)

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3

(where Z1 denotes Leu, Asp-Leu, or Leu-Asp-Leu, Z2 denotes Glu, Gln, or Asp, and Z3 denotes a set of 0 to 10 amino acid residues.)

(4) Asp-Leu-Z4-Leu-Arg-Leu

(where Z4 denotes Glu, Gln, or Asp.)

(5) α 1-Leu- β 1-Leu- γ 1-Leu

(6) α 1-Leu- β 1-Leu- γ 2-Leu

(7) α 1-Leu- β 2-Leu-Arg-Leu

(8) α 2-Leu- β 1-Leu-Arg-Leu

(where α 1 denotes Asp, Asn, Glu, Gln, Thr, or Ser, α 2 denotes Asn, Glu, Gln, Thr, or Ser, β 1 denotes Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His, β 2 denotes Asn, Arg, Thr, Ser, or His, γ 1 denotes Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp, and γ 2 denotes Gln, Asn, Thr, Ser, His, Lys, or Asp in formulae (5) to (8).)

[0013] In addition, the plant of the present invention provides significant improvement or reduction of productivity of a protein contained in seeds. Here, the expression "significant improvement or reduction" indicates that the plant of the present invention allows an increase or decrease in the seed protein content associated with a statistically significant

difference when compared in terms of material productivity with a plant in which the above chimeric protein is not expressed.

[0014] Meanwhile, according to the present invention, the above chimeric protein, the gene encoding the chimeric protein, an expression vector comprising the gene, and a transformant comprising the gene can be provided.

[0015] This description includes part or all of the contents as disclosed in the description and/or drawings of Japanese Patent Application No. 2009-135195, which is a priority document of the present application. Advantageous Effects of Invention

[0016] The seed protein content is improved or reduced in the plant of the present invention. Therefore, the use of the plant of the present invention enables mass production of a desired protein in seeds of the plant. Alternatively, seeds that exhibit a significant reduction in the content of a protein contained as an impurity or an allergen can be produced.

Description of Embodiments

[0017] The present invention will be described in detail as follows.

[0018] The plant of the present invention is a plant in which a chimeric protein obtained by fusing a predetermined transcription factor and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor is expressed, a plant in which a gene encoding a predetermined transcription factor is present as a result of gene introduction, or a plant in which an expression control region of an endogenous gene corresponding to the gene is modified. The plant of the present invention is found to exhibit significant improvement or reduction of the productivity of seed protein when compared with a wild-type plant. Specifically, the plant of the present invention is produced by causing a transcription factor to be expressed in the form of a chimeric protein with the functional peptide in a desired plant, introducing a gene encoding a predetermined transcription factor into a desired plant, or modifying an expression control region of an endogenous gene corresponding to the gene in a desired plant so as to significantly improve or reduce the protein content in seeds of the desired plant. Here, the expression level of the gene can be significantly increased compared with that in a wild-type plant by exogenously introducing a predetermined transcription factor into a plant or modifying an expression control region of an endogenous gene corresponding to the gene in a plant. The plant according to the present invention may be produced by causing the expression of the predetermined transcription factor in all plant tissues, or at least in some plant tissues. Here, the term "plant tissue(s)" is meant to include plant organ(s) such as leaves, stems, seeds, roots, and flowers.

[0019] Also, the term "expression control region" refers to a promoter region to which RNA polymerase binds and a region to which another transcription factor binds. A transcriptional regulatory region is preferably modified by substituting a promoter region, for example, among endogenous transcriptional regulatory regions with a promoter region that enables a higher expression level. In addition, when replacing, for example, a promoter region with a promoter region that enables a higher expression level, it becomes possible to cause overexpression of the predetermined transcription factor. Further, the term "overexpression" used herein also indicates a case in which a gene encoding a predetermined transcription factor present in a plant as a result of gene introduction is transcribed and thus is expressed at a level at which the gene can be confirmed as a transcription product.

[0020] In particular, preferably, the transactivation activity of a transcription factor is repressed in the plant of the present invention by fusing the factor with the above functional peptide. In other words, when a chimeric protein obtained by fusing a transcription factor with the functional peptide is expressed in the plant of the present invention, this preferably results in expression of transcription repression effects originally imparted to the functional peptide as a dominant trait.

[0021] A protein contained in a plant used herein may be any protein originally accumulated in seeds and any protein encoded by a gene exogenously introduced into the plant. In addition, genes to be exogenously introduced are introduced under control of, for example, a publicly known seed-specific expression promoter, thereby allowing efficient expression of the genes in seeds.

[0022] In particular, if the seed protein content increases, purification cost or transport cost can be reduced. Thus, such plant is highly industrially applicable. Meanwhile, a protein contained in seeds might become an impurity or allergen, depending on the usage of seeds. Therefore, if the productivity of a protein contained in seeds decreases, the impurity content or the allergen content also decreases. In such case, the seeds are highly industrially applicable.

[0023] Plants used herein are not particularly limited, and thus any plant can be used as a target plant. Examples of an available target plant include soybean, sesame, olive oil, coconut, rice, cotton, sunflower, corn, sugarcane, *Jatropha*, palm, tobacco, safflower, and rapeseed. Also, *Arabidopsis thaliana*, which has been widely used as a biological model for plant gene analysis and for which gene expression analysis methods have been established, can be used as a target plant.

[0024] In addition, transcription repression activity of a chimeric protein comprising a transcription factor is activity of recognizing a cis sequence that is recognized by the transcription factor or a cis sequence of a different transcription factor that is analogous to such a cis sequence so as to actively repress the expression of downstream genes. Thus, such chimeric protein can also be called a "transcriptional repressor." A method for causing a chimeric protein comprising

a transcription factor to have transcription repression activity is not particularly limited. However, the most preferable method may be a method for constructing a chimeric protein (fusion protein) by adding a repressor domain sequence or an SRDX sequence thereto.

[0025] In the above method, as a repressor domain sequence, a variety of amino acid sequences discovered by the present inventors, each of which constitutes a peptide capable of converting an arbitrary transcription factor into a transcriptional repressor, can be used. For example, the following can be referred to for a method using a repressor domain sequence: JP Patent Publication (Kokai) No. 2001-269177 A; JP Patent Publication (Kokai) No. 2001-269178 A; JP Patent Publication (Kokai) No. 2001-292776 A; JP Patent Publication (Kokai) No. 2001-292777 A; JP Patent Publication (Kokai) No. 2001-269176 A; JP Patent Publication (Kokai) No. 2001-269179 A; WO03/055903; Ohta, M., Matsui, K., Hiratsu, K., Shinshi, H. and Ohme-Takagi, M., The Plant Cell, Vol. 13, 1959-1968, August, 2001; and Hiratsu, K., Ohta, M., Matsui, K., or Ohme-Takagi, M., FEBS Letters 514(2002) 351-354. A repressor domain sequence can be excised from a Class II ERF (Ethylene Responsive Element Binding Factor) protein or a plant zinc finger protein (zinc finger protein such as *Arabidopsis thaliana* SUPERMAN protein). The sequence has a very simple structure.

[0026] Examples of a transcription factor constituting a chimeric protein to be expressed include transcription factors specified by AGI codes for *Arabidopsis thaliana* listed in tables 1 and 2. In addition, any transcription factor listed in table 1 causes a significant increase in seed protein content when a chimeric protein comprising the transcription factor and a repressor domain is expressed in a plant. Meanwhile, any transcription factor listed in table 2 causes a significant decrease in seed protein content when a chimeric protein comprising the transcription factor and a repressor domain is expressed in a plant.

[Table 1]

AGI code	Nucleotide sequence	Amino acid sequence
AT2G23760	SEQ ID NO: 1	SEQ ID NO: 2
AT1G18330	SEQ ID NO: 3	SEQ ID NO: 4
AT2G02070	SEQ ID NO: 5	SEQ ID NO: 6
AT1G12980	SEQ ID NO: 7	SEQ ID NO: 8
AT5G62380	SEQ ID NO: 9	SEQ ID NO: 10
AT4G23750	SEQ ID NO: 11	SEQ ID NO: 12
AT4G32800	SEQ ID NO: 13	SEQ ID NO: 14
AT1G24590	SEQ ID NO: 15	SEQ ID NO: 16
AT5G07690	SEQ ID NO: 17	SEQ ID NO: 18
AT1G71692	SEQ ID NO: 19	SEQ ID NO: 20
AT1G52150	SEQ ID NO: 21	SEQ ID NO: 22
AT3G25890	SEQ ID NO: 23	SEQ ID NO: 24
AT1G09540	SEQ ID NO: 25	SEQ ID NO: 26
AT5G22380	SEQ ID NO: 27	SEQ ID NO: 28
AT2G44940	SEQ ID NO: 29	SEQ ID NO: 30
AT5G41030	SEQ ID NO: 31	SEQ ID NO: 32
AT5G60970	SEQ ID NO: 33	SEQ ID NO: 34
AT5G35550	SEQ ID NO: 35	SEQ ID NO: 36
AT1G60240	SEQ ID NO: 37	SEQ ID NO: 38
AT2G23290	SEQ ID NO: 39	SEQ ID NO: 40
AT5G14000	SEQ ID NO: 41	SEQ ID NO: 42
AT1G19490	SEQ ID NO: 43	SEQ ID NO: 44

[Table 2]

AGI code	Nucleotide sequence	Amino acid sequence
AT1G32770	SEQ ID NO: 45	SEQ ID NO: 46
AT5G47220	SEQ ID NO: 47	SEQ ID NO: 48
AT1G56650	SEQ ID NO: 49	SEQ ID NO: 50
AT1G63910	SEQ ID NO: 51	SEQ ID NO: 52
AT3G15510	SEQ ID NO: 53	SEQ ID NO: 54
AT2G45680	SEQ ID NO: 55	SEQ ID NO: 56
AT2G31230	SEQ ID NO: 57	SEQ ID NO: 58
AT1G12260	SEQ ID NO: 59	SEQ ID NO: 60
AT3G61910	SEQ ID NO: 61	SEQ ID NO: 62
AT5G07310	SEQ ID NO: 63	SEQ ID NO: 64
AT3G14230	SEQ ID NO: 65	SEQ ID NO: 66
AT1G28160	SEQ ID NO: 67	SEQ ID NO: 68
AT1G69120	SEQ ID NO: 69	SEQ ID NO: 70
AT3G10490	SEQ ID NO: 71	SEQ ID NO: 72
AT5G61600	SEQ ID NO: 73	SEQ ID NO: 74
AT1G43160	SEQ ID NO: 75	SEQ ID NO: 76

[0027] Moreover, examples of a transcription factor that is introduced into a plant or in which a transcriptional regulatory region is modified include transcription factors specified by AGI codes for *Arabidopsis thaliana* listed in tables 3 and 4. In addition, any transcription factor listed in table 3 causes a significant increase in seed protein content when it is introduced into a plant or a transcriptional regulatory region thereof is modified. Any transcription factor listed in table 4 causes a significant decrease in seed protein content when it is introduced into a plant or a transcriptional regulatory region thereof is modified.

[Table 3]

AGI code	Nucleotide sequence	Amino acid sequence
AT3G04070	SEQ ID NO: 77	SEQ ID NO: 78
AT2G46770	SEQ ID NO: 79	SEQ ID NO: 80
AT5G35550	SEQ ID NO: 81	SEQ ID NO: 82

[Table 4]

AGI code	Nucleotide sequence	Amino acid sequence
AT1G10200	SEQ ID NO: 83	SEQ ID NO: 84

[0028] In addition, examples of a transcription factor constituting a chimeric protein or a transcription factor subjected to gene introduction or modification of an expression control region are not limited to amino acid sequences (shown in the even-numbered SEQ ID NOS: 1 to 84) listed in tables 1 to 4. Also, it is possible to use a transcription factor having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acid sequences with respect to any of the amino acid sequences. Here, the term "a plurality of amino acids" refers to 1 to 20, preferably 1 to 10, more preferably 1 to 7, further preferably 1 to 5, and particularly preferably 1 to 3 amino acids, for example. In addition, amino acid deletion, substitution, or addition can be performed by modifying a nucleotide sequence encoding any of the above transcription factors by a technique known in the art. Mutation can be introduced into a nucleotide sequence by a known technique such as the Kunkel method or

the Gapped duplex method or a method based thereon. For example, mutation is introduced with a mutagenesis kit using site-directed mutagenesis (e.g., Mutant-K or Mutant-G (both are trade names of Takara Bio)) or the like, or a LA PCR *in vitro* Mutagenesis series kit (trade name, Takara Bio). Also, a mutagenesis method may be: a method using a chemical mutation agent represented by EMS (ethyl methanesulfonate), 5-bromouracil, 2-aminopurine, hydroxylamine, N-methyl-N'-nitro-N nitrosoguanidine, or other carcinogenic compounds; or a method that involves radiation treatment or ultraviolet [UV] treatment typically using X-rays, alpha rays, beta rays, gamma rays, an ion beam, or the like.

[0029] Further, examples of a transcription factor constituting a chimeric protein or a transcription factor subjected to gene introduction or modification of an expression control region are not limited to *Arabidopsis thaliana* transcription factors listed in tables 1 to 4. Examples of such transcription factor can include transcription factors that function in a similar manner in non-*Arabidopsis thaliana* plants (e.g., the aforementioned plants) (hereinafter referred to as homologous transcription factors). These homologous transcription factors can be searched for using the genomic information of a search target plant based on amino acid sequences listed in tables 1 to 4 or the nucleotide sequences of individual genes if the plant genomic information has been elucidated. Homologous transcription factors can be identified by searching for amino acid sequences having, for example, 70% or higher, preferably 80% or higher, more preferably 90% or higher, and most preferably 95% or higher homology to the amino acid sequences listed in tables 1 to 4. Here, the value of homology refers to a value that can be found based on default setting using a computer equipped with a BLAST algorithm and a database containing gene sequence information.

[0030] In addition, a homologous gene can be identified by, when the plant genome information remains unclarified, extracting the genome from a target plant or constructing a cDNA library for a target plant and then isolating a genomic region or cDNA hybridizing under stringent conditions to at least some portions of the gene encoding any one of the transcription factors listed in tables 1 to 4. Here, the term "stringent conditions" refers to conditions under which namely a specific hybrid is formed, but a non-specific hybrid is never formed. For example, such conditions comprise hybridization at 45°C with 6 x SSC (sodium chloride/sodium citrate), followed by washing at 50°C to 65°C with 0.2-1 x SSC and 0.1% SDS. Alternatively, such conditions comprise hybridization at 65°C to 70°C with 1 x SSC, followed by washing at 65°C to 70°C with 0.3 x SSC. Hybridization can be performed by a conventionally known method such as a method described in J. Sambrook et al. Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory (1989).

[0031] A feature of causing the seed protein content to vary significantly (to be improved or reduced significantly) is imparted to the plant of the present invention by causing expression of the aforementioned chimeric protein comprising a transcription factor and a functional peptide in a plant, introducing the aforementioned gene encoding a transcription factor into a plant, or altering an expression control region of such gene in a plant.

[0032] In particular, a feature of causing the seed protein content to vary significantly (to be improved or reduced significantly) is imparted to the plant of the present invention by causing expression of a chimeric protein comprising a transcription factor of interest having repressed transactivation activity, further causing expression of transcription repression activity through recognition of a cis sequence homologous to a cis sequence recognized by the transcription factor of interest, and altering the specific affinity of the transcription factor of interest to that of another factor, nucleic acid, lipid, or carbohydrate. In the plant of the present invention, it is possible to create a chimeric protein comprising an endogenous transcription factor by modifying the endogenous transcription factor. Alternatively, it is also possible to introduce a gene encoding a chimeric protein into the plant so as to cause the gene to be expressed therein. For instance, it is preferable to use a method wherein a gene encoding a chimeric protein (fusion protein) obtained by fusing the aforementioned transcription factor and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor is introduced into a target plant to cause the chimeric protein (fusion protein) to be expressed in the plant.

[0033] The expression "transcription factor having repressed transactivation activity" used herein is not particularly limited. Such transcription factor has significantly lower transactivation activity than the original transcription factor. In addition, a "functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor" (sometimes referred to as a "transcription repressor converting peptide") is defined as a peptide having the function of causing an arbitrary transcription factor to have significantly reduced transactivation activity in comparison with the original transcription factor when the peptide is fused with the transcription factor to create a chimeric protein. Such "functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor" is not particularly limited. However, it is particularly preferable for the functional peptide to consist of an amino acid sequence known as a repressor domain sequence or an SRDX sequence. Examples of such transcription repressor converting peptide are described in detail in JP Patent Publication (Kokai) No. 2005-204657 A. Any example disclosed in such document can be used.

[0034] For example, a transcription repressor converting peptide consists of an amino acid sequence expressed by any one of the following formula (1) to (8).

[0035]

(1) X1-Leu-Asp-Leu-X2-Leu-X3

(where X1 denotes a set of 0 to 10 amino acid residues, X2 denotes Asn or Glu, and X3 denotes a set of at least 6 amino acid residues.)

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3

(where Y1 denotes a set of 0 to 10 amino acid residues, Y2 denotes Phe or Ile, and Y3 denotes a set of at least 6 amino acid residues.)

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3

(where Z1 denotes Leu, Asp-Leu, or Leu-Asp-Leu, Z2 denotes Glu, Gln, or Asp, and Z3 denotes a set of 0 to 10 amino acid residues.)

(4) Asp-Leu-Z4-Leu-Arg-Leu

(where Z4 denotes Glu, Gln, or Asp.)

(5) α 1-Leu- β 1-Leu- γ 1-Leu

(6) α 1-Leu- β 1-Leu- γ 2-Leu

(7) α 1-Leu- β 2-Leu-Arg-Leu

(8) α 2-Leu- β 1-Leu-Arg-Leu

(where α 1 denotes Asp, Asn, Glu, Gln, Thr, or Ser, α 2 denotes Asn, Glu, Gln, Thr, or Ser, β 1 denotes Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His, β 2 denotes Asn, Arg, Thr, Ser, or His, γ 1 denotes Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp, and γ 2 denotes Gln, Asn, Thr, Ser, His, Lys, or Asp in formulae (5) to (8).)

Transcription repressor converting peptide of formula (1)

[0036] The number of amino acid residues in the set denoted by "X1" may be 0 to 10 for the transcription repressor converting peptide of formula (1). In addition, types of specific amino acids corresponding to amino acid residues in the set denoted by X1 are not particularly limited. Any amino acid can be used. In view of ease of synthesis of the transcription repressor converting peptide of formula (1), it is preferable to minimize the length of the set of amino acid residues denoted by X1. Specifically, the number of amino acid residues in the set denoted by X1 is preferably not more than 5.

[0037] Similarly, the number of amino acid residues in the set denoted by X3 may be at least 6 for the transcription repressor converting peptide of formula (1). In addition, types of specific amino acids corresponding to amino acid residues in the set denoted by X3 are not particularly limited, and thus any amino acid may be used.

Transcription repressor converting peptide of formula (2)

[0038] As in the case of X1 for the transcription repressor converting peptide of formula (1), the number of amino acid residues in the set denoted by Y1 for the transcription repressor converting peptide of formula (2) may be 0 to 10. In addition, types of specific amino acids corresponding to amino acid residues in the set denoted by Y1 are not particularly limited, and thus any amino acid may be used. The number of specific amino acid residues in the set denoted by Y1 is preferably not more than 5.

[0039] Similarly, as in the case of X3 for the transcription repressor converting peptide of formula (1), the number of amino acid residues in the set denoted by Y3 for the transcription repressor converting peptide of formula (2) may be at least 6. In addition, types of specific amino acids corresponding to amino acid residues in the set denoted by Y3 are not particularly limited, and thus any amino acid may be used.

Transcription repressor converting peptide of formula (3)

[0040] For the transcription repressor converting peptide of formula (3), the set of amino acid residues denoted by Z1 contains 1 to 3 "Leu" amino acids. When it contains a single amino acid, Z1 denotes Leu. When it contains two amino acids, Z1 denotes Asp-Leu. When it contains 3 amino acids, Z1 denotes Leu-Asp-Leu.

[0041] Meanwhile, for the transcription repressor converting peptide of formula (3), the number of amino acid residues in the set denoted by Z3 may be 0 to 10. In addition, types of specific amino acids corresponding to amino acid residues in the set denoted by Z3 are not particularly limited, and thus any amino acid may be used. Specifically, the number of amino acid residues in the set denoted by Z3 is preferably not more than 5. Specific examples of an amino acid residue in the set denoted by Z3 include, but are not limited to, Gly, Gly-Phe-Phe, Gly-Phe-Ala, Gly-Tyr-Tyr, and Ala-Ala-Ala.

[0042] In addition, the number of amino acid residues consisting of a transcription repressor converting peptide as a whole of formula (3) is not particularly limited. However, in view of ease of synthesis, it is preferably not more than 20 amino acids.

Transcription repressor converting peptide of formula (4)

[0043] The transcription repressor converting peptide of formula (4) is a hexamer (6mer) consisting of 6 amino acid

residues. In addition, if the amino acid residue denoted by Z4 in the transcription repressor converting peptide of formula (4) is Glu, the amino acid sequence of the peptide corresponds to a region ranging from position 196 to position 201 of the amino acid sequence of the *Arabidopsis thaliana* SUPERMAN protein (SUP protein).

[0044] A chimeric protein (fusion protein) is created through fusion of any of the different transcription repressor converting peptides described above and any of the transcription factors described above so as to modify characteristics of the transcription factor. Specifically, a chimeric protein (fusion protein) is created through fusion of the transcription factor and the transcription repressor converting peptide, making it possible to modify the transcription factor into a transcriptional repressor or a negative transcriptional coactivator. In addition, it is possible to further convert a non-dominant transcriptional repressor into a dominant transcriptional repressor.

[0045] In addition, a chimeric protein (fusion protein) can be produced by obtaining a fusion gene of a polynucleotide encoding any transcription repressor converting peptide described above and a gene encoding a transcription factor. Specifically, a fusion gene is constructed by linking a polynucleotide encoding the transcription repressor converting peptide (hereinafter referred to as a "transcription repressor converting polynucleotide") and the gene encoding a transcription factor. The fusion gene is introduced into plant cells, thereby allowing production of a chimeric protein (fusion protein). The specific nucleotide sequence of the transcription repressor converting polynucleotide is not particularly limited. It is only necessary for the transcription repressor converting polynucleotide to comprise a nucleotide sequence corresponding to the amino acid sequence of the transcription repressor converting peptide in accordance with the genetic code of the peptide. In addition, if necessary, the transcription repressor converting polynucleotide may have a nucleotide sequence that serves as a linking site via which the transcription repressor converting polynucleotide is linked to a transcription factor gene. Further, if the amino acid reading frame of the transcription repressor converting polynucleotide does not match the reading frame of the transcription factor gene, the transcription repressor converting polynucleotide can comprise an additional nucleotide sequence that allows matching of both reading frames. Furthermore, the transcription repressor converting polynucleotide may comprise a variety of additional polypeptides such as a polypeptide having a linker function to link a transcription factor and a transcription repressor converting peptide and a polypeptide such as His, Myc, or Flag used for epitope labeling of a chimeric protein (fusion protein). Moreover, if necessary, the chimeric protein (fusion protein) may have a construct such as a sugar chain, an isoprenoid group, or the like as well as such polypeptide.

[0046] In addition, a conventionally known expression vector or the like can be used when the above gene encoding a transcription factor is introduced into plants.

[0047] A method for producing a plant is not particularly limited as long as it comprises a step of producing the above chimeric protein comprising a transcription factor and a transcription repressor converting peptide in a plant or a step of introducing the above gene encoding a transcription factor into a plant or modifying an expression control region of the gene. However, for example, a production method comprising steps such as an expression vector construction step, a transformation step, and a selection step can be used. Each step is specifically described below.

Expression vector construction step

[0048] The expression vector construction step is not particularly limited as long as it includes a step of constructing a recombinant expression vector containing the gene encoding a transcription factor, a transcription repressor converting polynucleotide, and a promoter. Also, the expression vector construction step is not particularly limited as long as it is a step of constructing a recombinant expression vector containing the gene encoding a transcription factor to be introduced and a promoter. As a vector serving as a mother body for a recombinant expression vector, various conventionally known vectors can be used. For example, plasmids, phages, cosmids, or the like can be used and such vector can be appropriately selected depending on plant cells into which it is introduced and introduction methods. Specific examples of such vector include pBR322, pBR325, pUC19, pUC119, pBluescript, pBluescriptSK, and pBI vectors. Particularly, when a method for introduction of a vector into a plant uses *Agrobacterium*, a pBI binary vector is preferably used. Specific examples of such pBI binary vector include pBIG, pBIN19, pBI101, pBI121, and pBI221.

[0049] A promoter used herein is not particularly limited as long as it can cause gene expression in plants. Any known promoter can be appropriately used. Examples of such promoter include a cauliflower mosaic virus 35S promoter (CaMV35S), various actin gene promoters, various ubiquitin gene promoters, a nopaline synthase gene promoter, a tobacco PR1a gene promoter, a tomato ribulose 1,5-bisphosphate carboxylase-oxidase small subunit gene promoter, a napin gene promoter, and an oleosin gene promoter. Of these, a cauliflower mosaic virus 35S promoter, an actin gene promoter, or a ubiquitin gene promoter can be more preferably used. The use of each of the above promoters enables strong expression of any gene when it is introduced into plant cells. The structure of a recombinant expression vector itself is not particularly limited as long as the promoter is linked to a fusion gene obtained by linking a gene encoding a transcription factor and a transcription repressor converting polynucleotide so as to cause expression of the gene and introduced into the vector. Also, the structure of a recombinant expression vector itself is not particularly limited as long as the promoter is linked to a gene encoding a desired transcription factor for gene introduction so as to cause expression

of the gene and introduced into the vector.

[0050] In addition, a recombinant expression vector may further contain other DNA segments, in addition to a promoter and the fusion gene or the gene encoding a transcription factor. Such other DNA segments are not particularly limited and examples thereof include a terminator, a selection marker, an enhancer, and a nucleotide sequence for enhancing translation efficiency. Also, the above recombinant expression vector may further have a T-DNA region. A T-DNA region can enhance efficiency for gene introduction particularly when the above recombinant expression vector is introduced into a plant using *Agrobacterium*.

[0051] A transcription terminator is not particularly limited as long as it has functions as a transcription termination site and may be any known transcription terminator. For example, specifically, a transcription termination region (Nos terminator) of a nopaline synthase gene, a transcription termination region (CaMV35S terminator) of cauliflower mosaic virus 35S, or the like can be preferably used. Of them, the Nos terminator can be more preferably used. In the case of the above recombinant vector, a phenomenon such that an unnecessarily long transcript is synthesized and that a strong promoter decreases the number of copies of a plasmid after introduction into plant cells can be prevented by arranging a transcription terminator at an appropriate position.

[0052] As a transformant selection marker, a drug resistance gene can be used, for example. Specific examples of such drug resistance gene include drug resistance genes against hygromycin, bleomycin, kanamycin, gentamicin, chloramphenicol, and the like. Transformed plants can be easily selected by selecting plants that can grow in medium containing the above antibiotics.

[0053] An example of a nucleotide sequence for increasing translation efficiency is an omega sequence from tobacco mosaic virus. This omega sequence is arranged in an untranslated region (5'UTR) of a promoter, so that the translation efficiency of the fusion gene can be increased. As such, the recombinant expression vector can contain various DNA segments depending on purposes.

[0054] A method for constructing a recombinant expression vector is not particularly limited. To an appropriately selected vector serving as a mother body, the above promoter, a fusion gene consisting of a gene encoding a transcription factor and a transcription repressor converting polynucleotide or a gene encoding a desired transcription factor for gene introduction, and, if necessary, the above other DNA segments may be introduced in a predetermined order. For example, a gene encoding a transcription factor and a transcription repressor converting polynucleotide are linked to construct a fusion gene, and then the fusion gene and the promoter (e.g., a transcription terminator according to need) are then linked to construct an expression cassette and then the cassette may be introduced into a vector.

[0055] In construction of a chimeric gene (fusion gene) and an expression cassette, for example, cleavage sites of DNA segments are prepared to have protruding ends complementary to each other and then performing a reaction with a ligation enzyme, making it possible to specify the order of the DNA segments. In addition, when an expression cassette contains a terminator, DNA segments may be arranged in the following order from upstream: a promoter, the fusion gene or the gene encoding a transcription factor, and a terminator. Also, reagents for construction of an expression vector (that is, types of restriction enzymes, ligation enzymes, and the like) are also not particularly limited. Hence, commercially available reagents can be appropriately selected and used.

[0056] Also, a method for replicating (a method for producing) the above expression vector is not particularly limited and conventionally known replication methods can be used herein. In general, such expression vector may be replicated within *Escherichia coli* as a host. At this time, preferred types of *Escherichia coli* may be selected depending on the types of vector.

Transformation step

[0057] The transformation step carried out in the present invention is a step of introducing the fusion gene or the gene encoding a transcription factor into plant cells using the above recombinant expression vector so as to cause the expression of the gene. A method for introducing such gene into plant cells (transformation method) using a recombinant expression vector is not particularly limited. Conventionally known appropriate introduction methods can be used depending on plant cells. Specifically, a method using *Agrobacterium* or a method that involves direct introduction into plant cells can be used, for example. As a method using *Agrobacterium*, a method described in the following can be employed, for example: Bechtold, E., Ellis, J. and Pelletier, G. (1993), In *Planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis plants*. C. R. Acad. Sci. Paris Sci. Vie, 316, 1194-1199; or Zyprian E, Kado CI, *Agrobacterium-mediated plant transformation by novel mini-T vectors in conjunction with a high-copy vir region helper plasmid*, Plant Molecular Biology, 1990, 15(2), 245-256.

[0058] As a method for directly introducing DNA comprising a recombinant expression vector and a target gene into plant cells, microinjection, electroporation, a polyethylene glycol method, a particle gun method, protoplast fusion, a calcium phosphate method, or the like can be employed.

[0059] Also, when a method for directly introducing DNA into plant cells is employed, DNA that can be used herein contains transcriptional units required for the expression of a target gene, such as a promoter and a transcription

terminator, and a target gene. Vector functions are not essential in such case. Moreover, a DNA that contains a protein coding region alone of a target gene having no transcriptional unit may be used herein, as long as it is integrated into a host's transcriptional unit and then the target gene can be expressed.

[0060] Examples of plant cells into which DNA comprising the above recombinant expression vector and a target gene or DNA containing no expression vector but a target gene DNA is introduced include cells of each tissue of plant organs such as flowers, leaves, and roots, calluses, and suspension-cultured cells. At this time, according to the plant production method of the present invention, an appropriate expression vector may be constructed as the above recombinant expression vector according to the type of plant to be produced or a versatile expression vector may be constructed in advance and then introduced into plant cells. That is to say, the plant production method of the present invention may or may not comprise a step of constructing a DNA for transformation using the recombinant expression vector.

Other steps and methods

[0061] The plant production method of the present invention needs to comprise at least the transformation step, and the method may further comprise a step of constructing the DNA for transformation using the recombinant expression vector. The method may further comprise other steps. Specifically, for example, a step of selecting an appropriate transformant from among transformed plants can be employed.

[0062] A selection method is not particularly limited. For example, selection may be carried based on drug resistance such as hygromycin resistance. Alternatively, selection may be carried out based on the protein content in plant seeds collected from cultivated transformants. For example, a method comprising collecting plant seeds, determining the protein content in the seeds according to a standard method, and comparing the protein content with the protein content in non-transformed plant seeds can be employed in a case in which selection is carried out based on protein content (see the Examples described below).

[0063] According to the plant production method of the present invention, the fusion gene or the gene encoding a transcription factor is introduced into a plant. This makes it possible to obtain an offspring plant having a significantly improved or reduced protein content in comparison with the plant via sexual reproduction or asexual reproduction. Also, plant cells or reproductive materials, such as seeds, fruits, stocks, calluses, tubers, cut ears, or lumps, may be obtained from the plant or an offspring plant thereof. The plant can be mass-produced therefrom based on such materials. Therefore, the plant production method of the present invention may comprise a reproduction step (mass production step) for reproducing a selected plant.

[0064] In addition, the plant of the present invention may include a matter comprising at least any one of an adult plant, plant cells, plant tissue, callus, and seeds. That is, according to the present invention, any matter in a state that allows it to eventually grow to become a plant can be regarded as a plant. In addition, plant cells include plant cells in various forms. Examples of such plant cells include suspension-cultured cells, protoplasts, and leaf sections. As a result of proliferation/differentiation of such plant cells, a plant can be obtained. In addition, a plant can be reproduced from plant cells by a conventionally known method depending on the types of plant cells. Therefore, the plant production method of the present invention may comprise a regeneration step of regenerating a plant from plant cells or the like.

[0065] In addition, the plant production method of the present invention is not limited to a method of transformation using a recombinant expression vector. A different method may be used. Specifically, for example, the chimeric protein (fusion protein) itself or a transcription factor (protein) can be administered to a plant. In this case, the chimeric protein (fusion protein) or a transcription factor (protein) can be administered to a young plant such that the seed protein content can be improved. In addition, a method of administration of a chimeric protein (fusion protein) or a transcription factor (protein) is not particularly limited, and a different known method can be used.

[0066] As described above, according to the present invention, it becomes possible to provide a plant for which the seed protein content has been caused to vary significantly (to be improved or reduced significantly) relative to the protein content in a wild-type plant by inducing expression of a chimeric protein comprising a predetermined transcription factor and any functional peptide described above or a predetermined transcription factor. When the chimeric protein is expressed in a plant, it might cause repression of transactivation activity of a target transcription factor or it might cause exhibition of transcription repression effects upon a sequence homologous to a cis sequence recognized by a target transcription factor. Further, in some cases, such chimeric protein functions to change the specific affinity of another factor, DNA, RNA, lipid, or carbohydrate having affinity to a target transcription factor or transcriptional coactivator. Alternatively, in some cases, it functions to cause a substance having no affinity to a target transcription factor to have improved affinity thereto. The following factors can be expressed in a similar manner in the plant of the present invention: a transcription factor that constitutes a chimeric protein; a transcription factor capable of recognizing a cis sequence homologous to a cis sequence recognized by the transcription factor; a transcription factor homologous to a transcription factor that constitutes a chimeric protein; other factors each having affinity to a transcription factor that constitutes a chimeric protein; and the like. However, the above effects of a chimeric protein allow suppression of gene expression to be controlled in a dominant-negative manner. Accordingly, the expression levels of gene groups involved in plant

growth and the expression levels of gene groups involved in protein production in seeds and/or gene groups involved in decomposition of a produced protein would vary in the plant of the present invention. This is thought to cause significant variation in seed protein content.

[0067] Here, significant variation in the seed protein content exists in a case in which the plant of the present invention exhibits an improvement of the protein amount over a wild-type plant while the single seed mass remains stable, a case in which the plant of the present invention is found to exhibit improvement of protein content with a significantly higher or lower level of single seed mass than that of a wild-type plant, or a case in which the plant of the present invention is found to exhibit improvement or reduction of seed protein content when compared with a wild-type plant. In any case, it corresponds to a variation in the amount of a protein produced by a single individual plant.

[0068] More specifically, if a chimeric protein comprising any transcription factor listed in table 1 is expressed in a plant, the protein content in seeds of the plant would be improved by approximately 20% or more compared with a wild-type plant. In addition, if a gene encoding any transcription factor listed in table 3 is introduced into a plant, the protein content in seeds of the plant would be improved by approximately 20% or more compared with a wild-type plant. Among the plants of the present invention, a plant confirmed to have increased protein content can be used for a method for producing a plant-derived protein. For example, a protein can be produced by cultivating the plant of the present invention, taking seeds therefrom, and collecting protein components from the obtained seeds. In particular, it can be said that the protein production method using the plant of the present invention is a method whereby high protein content in seeds can be achieved, resulting in excellent productivity. In other words, assuming that the number of cultivated plant individuals per unit area of cultivated acreage is stable and thus the amount of collected seeds is stable, the amount of protein produced per unit area of cultivated acreage can be remarkably improved with the use of the plant of the present invention. Therefore, production cost necessary for protein production can be significantly reduced with the use of the plant of the present invention.

Examples

[0069] The present invention is hereafter described in greater detail with reference to the following examples, although the technical scope of the present invention is not limited thereto.

[Example 1]

Transcription factor gene amplification

[0070] Each of the following transcription factors was subjected to PCR amplification of a coding region DNA fragment including or excluding a termination codon using the *Arabidopsis thaliana* cDNA library and primers described below:

At2g23760, At1g18330, At2g02070, At1g12980, At5g62380, At4g23750, At4g32800, At1g24590, At5g07690, At1g71692, At1g52150, At3g25890, At1g09540, At5g22380, At2g44940, At5g41030, At5g60970, At5g35550, At1g60240, At2g23290, At5g14000, At1g19490, At5g58900, At5g07580, At3g04070, At2g42830, At2g22200, At5g25190, At5g54230, At5g67300, At4g28140, At5g23260, At1g69490, At4g18390, At1g15360, At1g27370, At1g78080, At5g25390, At3g04060, At1g44830, At3g49850, At5g06100, At1g74840, At3g04070, At2g46770, At5g35550, At1g71030, At2g44840, At3g23220, At1g18570, At3g01530, At5g51190, At4g34410, At5g22290, At3g04420, At3g45150, At3g29035, At3g02150, At2g41710, At1g49120, At1g64380, At3g23230, At1g01010, At5g53290, At1g36060, At5g66300, At2g46310, At5g47390, At1g71030, At1g17520, At3g23220, At2g18060, At5g08070, At1g80580, At1g34190, At2g47520, At5g67000, At4g27950, At5g47230, At3g28910, At3g11280, At5g07680, At1g25470, At1g28520, At1g77450, At5g24590, At5g08790, At1g67260, At4g28530, At5g13910, At5g64530, At2g33710, At1g53230, At1g56010, At5g18560, At5g67580, At5g24520, At4g18390, At1g69690, At5g13330, At5g60970, At3g23220, At1g62700, At5g13330, At1g22985, At5g09330, At1g10200, At1g61110, At1g30210, At5g40330, At5g13180, At1g52880, At4g18450, At5g07580, At1g74930, At4g36160, At3g18550, At5g64750, At2g02450, At2g42400, At5g67300, At1g68800, At1g14510, At1g25580, At5g18270, At2g44840, At3g15500, At4g35580, At4g01550, At4g37750, At1g52890, At2g17040, At2g33480, At5g39610, At1g32770, At5g47220, At1g56650, At1g63910, At3g15510, At2g45680, At2g31230, At1g12260, At3g61910, At5g07310, At3g14230, At1g28160, At1g69120, At3g10490, At5g61600, At1g43160, At3g15210, At4g08150, and At1g10200. Note that a coding region DNA fragment including a termination codon was amplified for each of At3g04070, At2g46770, At5g35550, At1g71030, At2g44840, At4g18390, At1g69690, At5g13330, At5g60970, At3g23220, At3g15210, At4g08150, and At1g10200. PCR was carried out under conditions of 94°C for 1 minute, 47°C for 2 minutes, and elongation reaction at 74°C for 1 minute for 25 cycles. Next, each PCR product was isolated by agarose gel electrophoresis and collected.

[Table 5]				
AGI code	Fowerd primer	Nucleotide sequence	Reverse primer	Nucleotide sequence
At2g23760	GATGGGTTTAGCTACTACAAC TTCTTCTAT	SEQ ID NO: 85	AAAATCTCCAAAGTCTCTAACGGAGAAAGA	SEQ ID NO: 86
At1g18330	GATGGCCGCTGAGGATCGAAGTGAGGAACT	SEQ ID NO: 87	GCATATACGTGCTCTTTGGCTTTTCTTTTC	SEQ ID NO: 88
At2g02070	GATGGCTGCTTCTTCATCCTCCGCTGCTTC	SEQ ID NO: 89	GAAACTCGCATGATGGATTCCATAAGGTGG	SEQ ID NO: 90
At1g12980	AATGGAAAAAGCCTTGAGAACTTC	SEQ ID NO: 91	TCCCCACGATCTTCGGCAAGTACA	SEQ ID NO: 92
At5g62380	GATGGAAAGTCTCGCACACATTCCTCCCGG	SEQ ID NO: 93	CGTGTGTGTATTTTGAGCCCAAGAGTAGAA	SEQ ID NO: 94
At4g23750	ATGGAAGCGGAGAAGAAAATGG	SEQ ID NO: 95	AACAGCTAAAAGAGGATCCGAC	SEQ ID NO: 96
At4g32800	ATGGCGGATTCGTCTTCCGAC	SEQ ID NO: 97	GGGAAAATGTTCCAAGATTCG	SEQ ID NO: 98
At1g24590	ATGGAAGAAGCAATCATGAGAC	SEQ ID NO: 99	ATAATCATCATGAAAGCAATACTG	SEQ ID NO: 100
At5g07690	GATGTCAAGAAAGCCATGTTGTGTGGGAGA	SEQ ID NO: 101	TATGAAGTTCTTGTGCTCGTAATCTTGGCT	SEQ ID NO: 102
Atlg71692	GATGGCTCGTGGAAGATTCAAGCTTAAGAG	SEQ ID NO: 103	GAAGTGAATATTTCACTTGGCATTGTTAG	SEQ ID NO: 104
Atlg52150	GATGGCAATGTCTTGCAAGGATGGTAAGTT	SEQ ID NO: 105	CACAAAGGACCAATTGATGAACACAAAGCA	SEQ ID NO: 106
At3g25890	ATGGCTGAACGAAAGAAACGC	SEQ ID NO: 107	TGGGCACGCGATATTAAGAGG	SEQ ID NO: 108
At1g09540	GATGGGGAGACATTCTTGCTGTTACAAACA	SEQ ID NO: 109	AAGGGACTGACCAAAAGAGACGGCCATTCT	SEQ ID NO: 110
At5g22380	GATGGCCGATGAGGTCACAATCGGGTTTCG	SEQ ID NO: 111	AGGCCAAGTCAGCTGTTCCCAGTCCCACAT	SEQ ID NO: 112
At2g44940	ATGGCAAGACAAATCAACATAGAG	SEQ ID NO: 113	TTCAGATAGAAAAAACGGCTCTTC	SEQ ID NO: 114
At5g41030	ATGGTCATGGAGCCCAAGAAG	SEQ ID NO: 115	TGAACCATTTTCTCTGCACTC	SEQ ID NO: 116
At5g60970	ATGAGATCAGGAGAATGTGATG	SEQ ID NO: 117	AGAATCTGATTCAATTATCGCTAC	SEQ ID NO: 118
At5g35550	GATGGGAAAGAGAGCAACTACTAGTGTGAG	SEQ ID NO: 119	ACAAGTGAAGTCTCGGAGCCAATCTTCATC	SEQ ID NO: 120
At1g60240	GATGAAGTCAAGACGTGAACAATCAATCGA	SEQ ID NO: 121	TTTATAGTAACCTCGAATGTGCTGGGCCAA	SEQ ID NO: 122
At2g23290	GATGTCTGGTTGACCCGGAAGAAATGGA	SEQ ID NO: 123	CTCGATCCTACCTAATCCAATAAACTCTCT	SEQ ID NO: 124
At5g14000	GATGGAGGTGGAGAAGAGGATTGTAG	SEQ ID NO: 125	CTCATCAGCTGAGGTAGGAGGAG	SEQ ID NO: 126
At1g19490	GATGGAGTTGGAGCCTATATCATCGAGTTG	SEQ ID NO: 127	TCCGACCTGCATCCGACATTGACGGCCATG	SEQ ID NO: 128
At5g58900	GATGGAGGTTATGAGACCGTCGACGTCACA	SEQ ID NO: 129	TAGTTGAAACATTGTGTTTTGGGCGTCATA	SEQ ID NO: 130
At5g07580	ATGGCGAGTTTTGAGGAAAGC	SEQ ID NO: 131	AAATGCATCACAGGAAGATGAAG	SEQ ID NO: 132
At3g04070	GATGATAAGCAAGGATCCAAGATCGAGTTT	SEQ ID NO: 133	GCCTTGATATTGAAGGTGAGAACTCATCAT	SEQ ID NO: 134

(continued)				
AGI code	Fowerd primer	Nucleotide sequence	Reverse primer	Nucleotide sequence
At2g42830	GATGGAGGGTGGTGCGAGTAATGAAGTAGC	SEQ ID NO: 135	AACAAGTTGCAGAGGTGGTTGGTCTTGGTT	SEQ ID NO: 136
At2g22200	ATGGAACTGCTTCTTTCTTTC	SEQ ID NO: 137	AGAATTGGCCAGTTTACTAATTGC	SEQ ID NO: 138
At5g25190	ATGGCACGACCACAACAACGC	SEQ ID NO: 139	CAGCGTCTGAGTTGGTAAAACAG	SEQ ID NO: 140
At5g54230	GATGGGAAAATCTTCAAGCTCGGAGGAAAG	SEQ ID NO: 141	TGATAGATTCAAAGCATTATTATTATGATC	SEQ ID NO: 142
At5g67300	GATGGCTGATAGGATCAAAGGTCCATGGAG	SEQ ID NO: 143	CTCGATTCTCCCAACTCCAATTTGACTCAT	SEQ ID NO: 144
At4g28140	ATGGACTTTGACGAGGAGCTAAATC	SEQ ID NO: 145	AAAGAAAGGCCTCATAGGACAAG	SEQ ID NO: 146
At5g23260	GATGGGTAGAGGGAAGATAGAGATAAAGAA	SEQ ID NO: 147	ATCATTCTGGGCCGTTGGATCGTTTTGAAG	SEQ ID NO: 148
At1g69490	GATGGAAGTAACTTCCCAATCTACCCTCCC	SEQ ID NO: 149	AAACTTAAACATCGCTTGACGATGATGGTT	SEQ ID NO: 150
At4g18390	ATGATTGGAGATCTAATGAAG	SEQ ID NO: 151	GTTCTTGCCTTTACCCTTATG	SEQ ID NO: 152
At1g15360	ATGGTACAGACGAAGAAGTTCAG	SEQ ID NO: 153	GTTTGTATTGAGAAGCTCCTCTATC	SEQ ID NO: 154
At1g27370	GATGGACTGCAACATGGTATCTTCGTTCCC	SEQ ID NO: 155	GATGAAATGACTAGGGAAAGTGCCAAATAT	SEQ ID NO: 156
At1g78080	GATGGCAGCTGCTATGAATTTGTAC	SEQ ID NO: 157	AGCTAGAATCGAATCCCAATCG	SEQ ID NO: 158
At5g25390	ATGGTACATTGCAAGAAGTTCCG	SEQ ID NO: 159	GACCTGTGCAATGGATCCAG	SEQ ID NO: 160
At3g04060	GATGGTGGAAGAAGGCGGCGTAG	SEQ ID NO: 161	GCTAGTATATAAATCTTCCAGAAG	SEQ ID NO: 162
At1g44830	ATGGTGAAAACACTTCAAAGACAC	SEQ ID NO: 163	GCAGAAATTCCATAATCTGATATC	SEQ ID NO: 164
At3g49850	GATGGGAGCTCCAAAGCTGAAGTGGACACC	SEQ ID NO: 165	CCGAGTTTGGCTATGCATTCTATACTTCAC	SEQ ID NO: 166
At5g06100	GATGAGTTACACGAGCACTGACAGTGACCA	SEQ ID NO: 167	ACAAACTATTTCAAGTGATGGTAAGGTGAA	SEQ ID NO: 168
At1g74840	GATGGCCGACGGTAGTACTAGTTCTTCGGA	SEQ ID NO: 169	AGCGACTCCAATCGTGTGAATGCTGGATG	SEQ ID NO: 170
At3g04070	GATGATAAGCAAGGATCCAAGATCGAGTTT	SEQ ID NO: 171	CTAGCCTTGATATTGAAGGTGAGAACTCAT	SEQ ID NO: 172
At2g46770	GATGATGTCAAAATCTATGAGCATATC	SEQ ID NO: 173	TTATCCACTACCATTCGACACGTGACAAAA	SEQ ID NO: 174
At5g35550	GGGATGGGAAAGAGAGCAACTACTAGTGTGAGG	SEQ ID NO: 175	TCAACAAGTGAAGTCTCGGAGCCAATCTTC	SEQ ID NO: 176
At1g71030	GATGAACAAAACCCGCOTTCGTGCTCTCTC	SEQ ID NO: 177	TCATCGGAATAGAAGAAGCGTTTCTTGACC	SEQ ID NO: 178
At2g44840	ATGAGCTCATCTGATTCCGTTAATAAC	SEQ ID NO: 179	TTATATCCGATTATCAGAATAAGAAC	SEQ ID NO: 180
At3g23220	ATGAAATACAGAGGCGTACGAAAG	SEQ ID NO: 181	GCGGTTTGCGTCGTTACAATTG	SEQ ID NO: 182
At1g18570	GATGGTGCGGACACCGTGTTGCAAAGCTGA	SEQ ID NO: 183	TCCAAAATAGTTATCAATTTTCGTCAAACAA	SEQ ID NO: 184

[0071]

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At3g01530	GATGGAGACGACGATGAAGAAGAAAGGGAG	SEQ ID NO: 185	AATCACATGGTGGTCACCATTAAGCAAGTG	SEQ ID NO: 186
At5g51190	ATGGCTTCTTCACATCAACAACAG	SEQ ID NO: 187	AGTAACTACGAGTTGAGAGTGTC	SEQ ID NO: 188
At4g34410	ATGCATTATCCTAACAACAGAACCC	SEQ ID NO: 189	CTGGAACATATCAGCAATTGTATTTTC	SEQ ID NO: 190
At5g22290	GATGGACACGAAGGCGGTTGGAGTTTC	SEQ ID NO: 191	TTCTAGATAAAAACAACATTGCTATC	SEQ ID NO: 192
At3g04420	GATGGAGAATCCGGTGGGTTTAAG	SEQ ID NO: 193	TGTTCTTGAGATAGAAGAACATTGG	SEQ ID NO: 194
At3g45150	ATGGATTCGAAAAATGGAATTAAC	SEQ ID NO: 195	AACTGTGGTTGTGGCTGTTGTTG	SEQ ID NO: 196
At3g29035	GATGGATTACAAGGTATCAAGAAG	SEQ ID NO: 197	GAATTTCCAAACGCAATCAAGATTC	SEQ ID NO: 198
At3g02150	ATGAATATCGTCTCTTGAAAAGATG	SEQ ID NO: 199	TCACATATGGTGATCACTTCCTCTACTTG	SEQ ID NO: 200
At2g41710	GATGGCGTCGGTGTCTGTCGTC	SEQ ID NO: 201	TTTCTCTTGTTGGGAGGTAGCTG	SEQ ID NO: 202
At1g49120	ATGATCAGTTTCAGAGAAGAGAAC	SEQ ID NO: 203	TAAAACTTATCGATCCAATCAGTAG	SEQ ID NO: 204
At1g64380	ATGGAAGAAAGCAATGATATTTTTTC	SEQ ID NO: 205	ATTGGCAAGAACTTCCCAAATCAG	SEQ ID NO: 206
At3g23230	ATGGAGAGCTCAAACAGGAGC	SEQ ID NO: 207	TCTCTTCCTTTCTTCTGAATCAAG	SEQ ID NO: 208
At1g01010	GATGGAGGATCAAGTTGGGTTTGGG	SEQ ID NO: 209	ACCAACAAGAATGATCCAATAATG	SEQ ID NO: 210
At5g53290	ATGGACGAATATATTGATTTCCGAC	SEQ ID NO: 211	AGCAACTAATAGATCTGATATCAATG	SEQ ID NO: 212
At1g36060	ATGGCGGATCTCTTCGGTGG	SEQ ID NO: 213	CGATAAAATTGAAGCCCAATCTATC	SEQ ID NO: 214
At5g66300	GATGATGAAGGTTGATCAAGATTATTCGTG	SEQ ID NO: 215	GTCTTCTCCACTCATCAAAAATTGAGACGC	SEQ ID NO: 216
At2g46310	ATGAAAAGCCGAGTGAGAAAATC	SEQ ID NO: 217	TTACTTATCCAACAAATGATCTTGG	SEQ ID NO: 218
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At1g71030	GATGAACAAAACCCGCCTTCGTGCTCTCTC	SEQ ID NO: 221	TCGGAATAGAAGAAGCGTTTCTTGACCTGT	SEQ ID NO: 222
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At1g34190	GATGGCGGATTCTTCACCCGATTGG	SEQ ID NO: 233	GTCTTTCAAGAGAAGACTTCTACC	SEQ ID NO: 234
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At1g28520	GATGACGGGGAAGCGATCAAAGAC	SEQ ID NO: 251	GGGGATATAATAGTCGCTTAGATTTTC	SEQ ID NO: 252
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At5g24590	GATGAAAGAAGACATGGAAGTACTATC	SEQ ID NO: 255	TGCGACTAGACTGCAGACCGACATC	SEQ ID NO: 256
At5g08790	GATGAAGTCGGAGCTAAATTTACCAGCTGG	SEQ ID NO: 257	CCCCTGTGGAGCAAACTCCAATTCAAGAA	SEQ ID NO: 258
At1g67260	ATGTCGTCTTCCACCAATGAC	SEQ ID NO: 259	GTTTACAAAAGAGTCTTGAATCC	SEQ ID NO: 260
At4g28530	GATGGGTTTGAAAGATATTGGGTCC	SEQ ID NO: 261	TTGGAAAGCGAGGATATTTTCGGTC	SEQ ID NO: 262
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At2g33710	ATGCATAGCGGGAAGAGACCTC	SEQ ID NO: 267	TTTTCGTCGTTTGTGGATACTAATG	SEQ ID NO: 268
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[0072]

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At5g40330	ATGAGAATGACAAGAGATGGAAAAG	SEQ ID NO: 303	AAGGCAATACCCATTAGTAAATCCATCATAG	SEQ ID NO: 304
At5g13180	GATGGATAATGTCAAACCTTGTTAAGAATGG	SEQ ID NO: 305	TCTGAAACTATTGCAACTACTGGTCTCTTC	SEQ ID NO: 306
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At4g18450	ATGGCTTTTGGAATATCCAAG	SEQ ID NO: 309	AAAAGAAGATAATAACGTCTCC	SEQ ID NO: 310
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At5g18270	GATGGCGGTTGTGGTTGAAGAAGG	SEQ ID NO: 333	GAAGTCCCACAAGTCCCCCCTC	SEQ ID NO: 334
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At3g15500	GATGGGTCTCCAAGAGCTTGACCCGTTAGC	SEQ ID NO: 337	AATAAACCCGAACCCACTAGATTGTTGACC	SEQ II NO: 338

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At1g52890	GATGGGTATCCAAGAACTGACCCGTTAAC	SEQ ID NO: 345	CATAAACCCAAACCCACCAACTTGCCCCGA	SEQ ID NO: 346
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At2g33480	GATGGAGAAGAGGAGCTCTATTAACACAG	SEQ ID NO: 349	TAGAAACAAACAAAACCTATTTTCCCGATA	SEQ ID NO: 350
At5g39610	GATGGATTACGAGGCATCAAGAATC	SEQ ID NO: 351	GAAATCCAAACGCAATCCAATTC	SEQ ID NO: 352
At1g32770	GATGGCTGATAATAAGGTCAATCTTTGAT	SEQ ID NO: 353	TACAGATAAATGAAGAAGTGGGTCTAAAGA	SEQ ID NO: 354
At5g47220	GATGTACGGACAGTGCAATATAGAATCCG	SEQ ID NO: 355	TGAAACCAATAACTCATCAACACGTGT	SEQ ID NO: 356
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At1g63910	GATGGGTCATCACTCATGCTGCAACCAGCA	SEQ ID NO: 359	AAACGAAGAAGGGAAAGAAGAAGATAAGGC	SEQ ID NO: 360
At3g15510	GATGGAGAGCACCGATTCTTCCGGTGGTCC	SEQ ID NO: 361	AGAAGAGTACCAATTTAAACCGGGTAATTG	SEQ ID NO: 362
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At2g31230	ATGTATTCATCTCCAAGTTCTTGG	SEQ ID NO: 365	ACATGAGCTCATAAGAAGTTGTTC	SEQ ID NO: 366
At1g12260	GATGAATTCATTTCCACGTCCCTCCGGG	SEQ ID NO: 367	CTTCCATAGATCAATCTGACAACTCGAAGA	SEQ ID NO: 368
At3g61910	GATGAACATATCAGTAAACGGACAGTCACA	SEQ ID NO: 369	TCCACTACCGTTCAACAAGTGGCATGTCGT	SEQ ID NO: 370
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At1g28160	ATGGAGTTCAATGGTAATTTGAATG	SEQ ID NO: 375	TTGGTAGAAGAATGTGGAGGG	SEQ ID NO: 376
At1g69120	GATGGGAAGGGGTAGGGTTCAATTGAAGAG	SEQ ID NO: 377	TGCGGCGAAGCAGCCAAGGTTGCAGTTGTA	SEQ ID NO: 378
At3g10490	GATGGGTCGCGAATCTGTGGCTGTTG	SEQ ID NO: 379	TTGTCCATTAGCATTGTTCTTCTTG	SEQ ID NO: 380
At5g61600	ATGGCAACTAAACAAGAAGCTTTAG	SEQ ID NO: 381	AGTGACGGAGATAACGGAAAAG	SEQ ID NO: 382
At1g43160	ATGGTGTCTATGCTGACTAATG	SEQ ID NO: 383	ACCAAAAGAGGAGTAATTGTATTG	SEQ ID NO: 384
At3g15210	GGGGATGGCCAAGATGGGCTTGAAAC	SEQ ID NO: 385	TCAGGCCTGTTCCGATGGAGGAGGC	SEQ ID NO: 386
At4g08150	ATGGAAGAATACCAGCATGACAAC	SEQ ID NO: 387	TCATGGACCGAGACGATAAGGTCC	SEQ ID NO: 388
At1g10200	GGGATGGCGTTCGCAGGAACAACCCAGAAATG	SEQ ID NO: 389	TTAAGCAGCGACGACTTTGTCC	SEQ ID NO: 390

Production of improved transcription factors

[0073] In order to add a repressor domain sequence to the 3' terminal of a transcription factor gene encoded by a coding region DNA fragment excluding a termination codon, p35SSXG, which is a vector having an SmaI site and a repressor domain sequence (amino acid sequence: GLDLLELRGLGFA (SEQ ID NO: 391)) downstream of a CaMV35S promoter, was used. In order to link a transcription factor gene sequence and a repressor domain sequence, p35SSXG was cleaved with SmaI. Each PCR amplification fragment encoding the relevant transcription factor obtained above was separately inserted at the cleavage site. Thus, vectors (each denoted by p35SSXG(TFs)) were produced. Here, each vector is denoted by p35SSXG(TFs), provided that "TFs" represents the AGI code for each transcription factor. For example, a vector having the transcription factor specified by At2g23760 is denoted by p35SSXG(At2g23760). Also, in the descriptions below, "TFs" is used in a similar manner to denote vectors and the like.

Construction of improved transcription factor expression vectors

[0074] pBCKH was used as a binary vector for gene introduction into plants with *Agrobacterium*. This vector was obtained by incorporating a cassette of the Gateway vector conversion system (Invitrogen) into the HindIII site of pBIG (Hygr) (Nucleic Acids Res. 18,203 (1990)). In order to incorporate an improved transcription factor gene sequence into the vector, 181 types of p35SSXG(TFs) were each separately mixed with the vector, followed by a recombination reaction using GATEWAY LR clonase (Invitrogen). Thus, vectors (each denoted by pBCKH-p35SSXG(TFs)) were produced.

[0075] In addition, for each transcription factor encoded by the relevant coding region DNA fragment including a termination codon, the gene encoding the transcription factor was selected for introduction. Thus, vectors, in each of which the relevant DNA fragment was linked downstream of a 35S promoter in the manner described above, were produced.

Introduction of improved transcription factor gene expression vectors and transcription factor expression vectors into plants

[0076] *Arabidopsis thaliana* (Columbia (Col-0)) was used as a plant for introduction of a transcription factor or an improved transcription factor. Gene introduction was carried out in accordance with "Transformation of *Arabidopsis thaliana* by vacuum infiltration" (<http://www.bch.msu.edu/pamgreen/protocol.htm>). Note that each plant was infected only by immersing it in an *Agrobacterium* bacterial liquid without conducting depressurization treatment. Specifically, a transcription factor expression vector or an improved transcription factor expression vector (pBCKH-p35SSXG(TFs)) was introduced into the soil bacterium (*Agrobacterium tumefaciens*) strain (GV3101 (C58C1Rifr) pMP90 (Gmr), Koncz and Schell 1986)) by electroporation. For each vector, gene-transfected bacterial cells were cultured in 1 liter of a YEP medium containing antibiotics (kanamycin (Km): 50 µg/ml; gentamicin (Gm): 25 µg/ml; and rifampicin (Rif): 50 µg/ml)) until OD600 became 1. Subsequently, bacterial cells were recovered from each culture solution and suspended in 1 liter of an infection medium (an infiltration medium containing 2.2 g of an MS salt, 1X B5 vitamins, 50 g of sucrose, 0.5 g of MES, 0.044 µM of benzylaminopurine, and 400 µl of Silwet per liter (pH 5.7)).

[0077] *Arabidopsis thaliana* plants cultivated for 14 days were immersed in each solution for 1 minute for infection. Thereafter, the plants were continuously cultivated to result in seed setting. The collected seeds (T1 seeds) were sterilized in a solution containing 50% bleach and 0.02% Triton X-100 for 7 minutes, rinsed 3 times with sterilized water, and seeded on a sterilized hygromycin selection medium (containing a 4.3 g/l MS salt, 0.5% sucrose, 0.5 g/l MES (pH 5.7), 0.8% agar, 30 mg/l hygromycin, and 250 mg/l vancomycin). Five to ten lines of the transformed plants (T1 plants) growing on the hygromycin plate were selected for each improved transcription gene and transplanted into pots (each with a diameter of 50 mm) containing vermiculite mixed soil. Then, the plants were cultivated under conditions of 22°C for 16 hours in the light and 8 hours in the dark at a light intensity ranging from about 60 to 80 µE/cm². Thus, seeds (T2 seeds) were obtained.

Analysis of T2 seeds

[0078] Forty seeds were weighed and put into a 1.5-ml PP microtest tube for each of the transformants and wild-type *Arabidopsis thaliana*, which had been transfected with the relevant improved transcription factor or transcription factor. Further, a Tungsten Carbide Bead (3 mm) (QIAGEN) was put into each tube, followed by disruption by shaking at a frequency of 1/30 for 1 minute using a Mixer Mill MM 300 (Qiagen). After disruption, 50 µl of extraction buffer (62.5 mM Tris-HCl, 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol) was added thereto, followed by another instance of disruption by shaking for 1 minute. After disruption, each tube was allowed to stand on ice for 10 minutes, followed by centrifugation at 15000 rpm for 10 minutes. Each obtained supernatant was subjected to quantitative protein determination.

[0079] Quantitative protein determination for the prepared extracts was carried out using RC DC Protein Assay Kits

(Bio-Rad) according to the manufacturer's instructions. The protein concentration was determined based on a calibration curve derived from BSA (bovine serum albumin).

[0080] In addition, 34 individuals of the wild strain (Col-0) were cultivated and seeds were collected from each individual. The protein content was determined for each line by quantitative analysis. Then, the average protein content was obtained. Thereafter, the average protein content of each transgenic individual was compared with the average protein content of the wild strain. The protein content increase rate for each gene-transfected line and the t-test P value were determined. Each line was found to exhibit improvement or reduction of seed protein content by 20% or more when compared with a wild-type strain. However, the P value was found to be 5% or less for each comparison.

[0081] Table 6 lists the analysis results for each line that was found to exhibit improvement of seed protein content by 20% or more as a result of introduction of the relevant improved transcription factor when compared with the wild-type strain. Table 7 lists the analysis results for each line that were found to exhibit improvement of seed protein content by 20% or more as a result of introduction of the gene encoding the relevant transcription factor when compared with the wild-type strain.

[Table 6]

	AGI code	Reference number	Protein content (%)	Increase-decrease rate (%)
		WT(Col-0)	16.3%	-
1	At2g23760	HR0530	25.7%	57.5%
2	At1g18330	CR711	25.2%	54.2%
3	At2g02070	HR0489	23.9%	46.3%
4	At1g12980	TP120	22.6%	38.7%
5	At5g62380	CR604	22.5%	38.2%
6	At4g23750	CR034	21.8%	33.7%
7	At4g32800	CR504	21.6%	32.1%
8	At1g24590	CR019	21.4%	31.3%
9	At5g07690	HR0040	21.2%	29.8%
10	At1g71692	CR412	21.0%	28.9%
11	At1g52150	HR0611	20.9%	27.9%
12	At3g25890	CR029	20.4%	24.9%
13	At1g09540	CR705	20.4%	24.8%
14	At5g22380	CR229	20.3%	24.5%
15	At2g44940	CR505	20.3%	24.1%
16	At5g41030	CR131	20.2%	23.6%
17	At5g60970	CR116	20.1%	23.1%
18	At5g35550	CR701	20.0%	22.4%
19	Atlg60240	CR623	19.9%	22.2%
20	At2g23290	HR0018	19.9%	21.8%
21	At5g14000	CR223	19.7%	20.9%
22	At1g19490	HR0001	19.6%	20.2%

[Table 7]

	AGI code	Reference number	Protein content (%)	Increase-decrease rate (%)
		WT(Col-0)	16.3%	-
1	At3g04070	CR312	22.1%	35.7%
2	At2g46770	CR308	21.0%	28.6%
3	At5g35550	CR903	21.0%	28.5%

[0082] Table 8 lists the analysis results for each line that was found to exhibit reduction of seed protein content by 20% or more as a result of introduction of the relevant improved transcription factor when compared with the wild-type strain. Table 9 lists the analysis results for each line that were found to exhibit reduction of seed protein content by 20%

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or more as a result of introduction of the gene encoding the relevant transcription factor when compared with a wild-type strain.

[Table 8]

	AGI code	Reference number	Protein content (%)	Increase-decrease rate (%)
		WT(Col-0)	16.3%	0.0%
1	At1g32770	CR250	12.8%	-21.6%
2	At5g47220	TP100	12.8%	-21.6%
3	At1g56650	TP107	12.7%	-22.2%
4	At1g63910	HR1722	12.5%	-23.5%
5	At3g15510	CR245	12.5%	-23.7%
6	At2g45680	CR121	12.4%	-24.3%
7	At2g31230	CR006	12.2%	-25.2%
8	At1g12260	CR232	12.1%	-25.6%
9	At3g61910	CR601	11.9%	-27.3%
10	At5g07310	CR008	11.9%	-27.3%
11	At3g14230	CR014	11.9%	-27.3%
12	At1g28160	CR020	11.8%	-27.4%
13	At1g69120	CR404	11.8%	-27.6%
14	At3g10490	CR220	11.8%	-27.7%
15	At5g61600	CR001	11.5%	-29.7%
16	At1g43160	CR015	10.9%	-33.1%

[Table 9]

	AGI code	Reference number	Protein content (%)	Increase-decrease rate (%)
		WT(Col-0)	16.3%	0.0%
1	At1g10200	TP106	13.0%	-20.5%

[0083] In addition, T2 seeds of a line (HR0530) (into which the improved transcription factor (At2g23760) listed in fig. 6 with the results demonstrating the largest increase in protein content had been introduced) were cultivated, followed by re-evaluation of the protein content. Table 10 lists the results. As shown in table 10, it was also possible to confirm an increase in protein content for T3 seeds. In particular, the protein content was found to be up to 43% higher than that of the wild-type line. In addition, it was confirmed that SDS-PAGE caused no changes in seed protein composition (not shown).

[Table 10]

	Protein concentration (mg/ml)	Increasing rate (%)	Protein content (%)	Increasing rate (%)	Total protein amount (mg)	Increasing rate (%)
Average of WT (10 individuals)	1.6		26.6		71.8	
HR0530-23-4	2.4	46.3	36.9	38.5	90.7	26.3
HR0530-23-10	2.3	43.0	39.4	48.2	130.1	81.1
HR0530-23-8	2.3	39.6	39.0	46.7	103.9	44.7

[0084] As described above, the expression of SRDX-added chimeric proteins formed with 141 types of transcription factors was induced in this analysis. Results showed that the seed storage protein content increased by 20% or more as a result of expression of 22 types of chimeric proteins (accounting for 15.6% of the analyzed transcription factors),

while the seed storage protein content decreased by 20% or more as a result of expression of 16 types of chimeric proteins (accounting for 11.3% of the analyzed transcription factors). That is to say, the seed storage protein content was found to have remarkably increased or decreased as a result of expression of approximately 27% of the chimeric proteins. In other words, it was found that approximately 73% of the transcription factors (e.g., At3g23220, At1g18570, At3g01530, At5g51190, At4g34410, At5g22290, and At3g04420) subjected to the experiments in the Examples do not cause remarkable changes in seed protein content even when a chimeric protein comprising such a transcription factor and a repressor domain is expressed or such a transcription factor is overexpressed.

[0085] As described above, the Examples revealed that the seed protein content can be significantly modified by causing expression of a particular transcription factor fused with a repressor domain, introducing a gene encoding a particular transcription factor, or modifying an expression control region of such gene.

[0086] In addition, in order to increase or decrease the seed protein content with the use of the above functionally improved transcription factors, it is expected that it will become possible to further modify the storage protein content to a remarkable extent with the simultaneous use of transcription factors and a known method for modifying a seed storage protein by modifying the nitrogen metabolic pathway, the fatty acid metabolic pathway, or transcription factors.

[0087] All publications, patents, and patent applications cited herein are incorporated herein by reference in their entirety.

SEQUENCE LISTING

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 Toyota Central R&D Labs., Inc.
 National Institute of Advanced Industrial Science and Technology

<120> A gene capable of increasing a content of protein in seeds and use thereof

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 aac tca act ctt cat atg tta tta cca aat cat cat caa ggt ttt gct 336
 Asn Ser Thr Leu His Met Leu Leu Pro Asn His His Gln Gly Phe Ala
 100 105 110
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 Phe Thr Asp Glu Asn Thr Met Gln Pro Gln Gln Gln Gln His Phe Thr
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 Trp Pro Ser Ser Ser Ser Asp His His Gln Asn Arg Asp Met Ile Gly
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	Ala Val Asp Gly Thr Ser Ser Ser Ser Asn Ala Ser Ala His His His	
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	Gln Phe Asn Gln Phe Lys Asn Leu Leu Leu Glu Asn Ser Ser Ser Gln	
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	His His His His Gln Val Val Gly His Phe Gly Ser Ser Ser Ser Ser	
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	Pro Met Ala Ala Ser Ser Ser Ile Gly Gly Ile Tyr Thr Leu Arg Asn	
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	Ser Lys Tyr Thr Lys Pro Ala Gln Glu Leu Leu Glu Glu Phe Cys Ser	
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35	gtt gga aga gga cat ttc aag aag aac aaa ctt agt agg aac aac tca	816
	Val Gly Arg Gly His Phe Lys Lys Asn Lys Leu Ser Arg Asn Asn Ser	
	260 265 270	
40	aac cct aat act acc ggt gga gga gga ggc gga ggg tcc tcg tca tcg	864
	Asn Pro Asn Thr Thr Gly Gly Gly Gly Gly Gly Ser Ser Ser Ser	
	275 280 285	
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	Ala Gly Thr Ala Asn Asp Ser Pro Pro Leu Ser Pro Ala Asp Arg Ile	
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	Asp Arg Arg Tyr Asn His Tyr Cys Glu Gln Met Gln Met Val Val Asn	
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	Ser Phe Asp Gln Val Met Gly Tyr Gly Ala Ala Val Pro Tyr Thr Thr	
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	Leu Ala Gln Lys Ala Met Ser Arg His Phe Arg Cys Leu Lys Asp Ala	
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	Val Ala Val Gln Leu Lys Arg Ser Cys Glu Leu Leu Gly Asp Lys Glu	
	370 375 380	
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	Ala Ala Gly Ala Ala Ser Ser Gly Leu Thr Lys Gly Glu Thr Pro Arg	
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	Ser	His	Gly	Gly	Ser	Asp	Ala	Phe	Thr	Val	Ala	Thr	Cys	Gln	Gln	Asp	
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	ggg	acc	aaa	cag	act	ggg	gac	gtg	tct	ctt	acg	ctt	ggg	cta	cgc	cac	1824
	Gly	Thr	Lys	Gln	Thr	Gly	Asp	Val	Ser	Leu	Thr	Leu	Gly	Leu	Arg	His	
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	Tyr	Pro	Gly	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Glu	Ile	Leu	Asp	Gln		65	70	75	80
20	Ser	Thr	Lys	Gln	Leu	Leu	Glu	Gln	Gln	Asn	Arg	His	Asn	Asn	Asn	Asn	85	90	95	
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	Phe	Thr	Asp	Glu	Asn	Thr	Met	Gln	Pro	Gln	Gln	Gln	Gln	His	Phe	Thr	115	120	125	
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	Ser	Leu	Ala	Ala	Ala	Lys	Ala	Glu	Glu	Tyr	Arg	Ser	Ile	Tyr	Cys	Ala	165	170	175	
40	Ala	Val	Asp	Gly	Thr	Ser	Ser	Ser	Ser	Asn	Ala	Ser	Ala	His	His	His	180	185	190	
45	Gln	Phe	Asn	Gln	Phe	Lys	Asn	Leu	Leu	Leu	Glu	Asn	Ser	Ser	Ser	Gln	195	200	205	
	His	His	His	His	Gln	Val	Val	Gly	His	Phe	Gly	Ser	Ser	Ser	Ser	Ser	210	215	220	
50	Pro	Met	Ala	Ala	Ser	Ser	Ser	Ile	Gly	Gly	Ile	Tyr	Thr	Leu	Arg	Asn	225	230	235	240
55	Ser	Lys	Tyr	Thr	Lys	Pro	Ala	Gln	Glu	Leu	Leu	Glu	Glu	Phe	Cys	Ser	245	250	255	

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 Asp Arg Arg Tyr Asn His Tyr Cys Glu Gln Met Gln Met Val Val Asn
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 Met Gly Met Met Glu Gln Glu Ala Trp Arg Pro Gln Arg Gly Leu Pro
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 Gly Leu Ser Arg Asn Gln Val Ser Asn Trp Phe Ile Asn Ala Arg Val
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	Ala	Ala	Arg	Lys	Arg	Phe	Arg	Tyr	Phe	Leu	Met	Ala	Ala	Glu	Asp	Arg	
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	Ser	Glu	Glu	Leu	Ser	Ser	Asn	Val	Glu	Asn	Gly	Ser	Cys	Asn	Ser	Asn	
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50	Glu	Gly	Ile	Asn	Pro	Glu	Thr	Ser	Ser	His	Trp	Ile	Glu	Asn	Val	Val	
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	Lys	Val	Arg	Lys	Pro	Tyr	Thr	Val	Thr	Lys	Gln	Arg	Glu	Lys	Trp	Ser	
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	Glu	Glu	Glu	His	Asp	Arg	Phe	Leu	Glu	Ala	Ile	Lys	Leu	Tyr	Gly	Arg	

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3A

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AD

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5A

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	Asp	Asp	Gln	Ser	His	Leu	Leu	Pro	Pro	Asn	Ser	Ser	Ala	Ala	Ala	Pro	

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20	ttc ata tgt gaa gta tgc aac aaa ggg ttt caa aga gaa cag aat cta Phe Ile Cys Glu Val Cys Asn Lys Gly Phe Gln Arg Glu Gln Asn Leu 85 90 95	288		
25	caa ctt cac cga aga gga cac aat ctt cca tgg aag ctc aaa cag aaa Gln Leu His Arg Arg Gly His Asn Leu Pro Trp Lys Leu Lys Gln Lys 100 105 110	336		
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40	aag aaa cat tat tac cgt aaa cac ggt gaa aag aag tgg aaa tgc gat Lys Lys His Tyr Tyr Arg Lys His Gly Glu Lys Lys Trp Lys Cys Asp 145 150 155 160	480		
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65	cat cac ttc ccg tac gga caa aac aca aac aac tcc aac aac aac gct His His Phe Pro Tyr Gly Gln Asn Thr Asn Asn Ser Asn Asn Asn Ala 225 230 235 240	720		
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25	ctc tcc gga aac aac gga gtt act tct gcc aca agt aac cct aat gct Leu Ser Gly Asn Asn Gly Val Thr Ser Ala Thr Ser Asn Pro Asn Ala 355 360 365	1104
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40	ctc ttc cct aac aat ctg atg agc tcg gca gat aga att agc tca gga Leu Phe Pro Asn Asn Leu Met Ser Ser Ala Asp Arg Ile Ser Ser Gly 405 410 415	1248
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	Phe Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln	
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	His Gly Asn Ser Arg Glu Arg Val Gly Ser Ser Ser Asp Ser Ala Asp	
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10	aga agc agc atg aat gtg aat acc gga ggt ggt ccg gca agt act tca	1776
	Arg Ser Ser Met Asn Val Asn Thr Gly Gly Gly Pro Ala Ser Thr Ser	
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	Gln Leu His Arg Arg Gly His Asn Leu Pro Trp Lys Leu Lys Gln Lys	
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	Lys Lys His Tyr Tyr Arg Lys His Gly Glu Lys Lys Trp Lys Cys Asp	
	145 150 155 160	
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	225					230					235					240
	Ser	Ser	Met	Ile	Leu	Gly	Leu	Ser	His	Met	Gly	Ala	Pro	Gln	Asn	Leu
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20	Asp	His	Gln	Pro	Gly	Asp	Val	Leu	Arg	Leu	Gly	Ser	Gly	Gly	Gly	Gly
				260					265					270		
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	385					390					395					400
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Ser Thr Ser Ser Asn Asn Asn Asn Gly Ser Asn Thr Asn Asn Asn Asn
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10 Asn Ala Ser Ser Ile Leu Arg Ser Phe Gly Ser Gly Ile Tyr Gly Glu
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Asn Glu Ser Asn Leu Gln Asp Leu Met Asn Ser Phe Ser Asn Pro Gly
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25 530 535 540

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30 His Gly Asn Ser Arg Glu Arg Val Gly Ser Ser Ser Asp Ser Ala Asp
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Pro Asn Pro Leu Thr Lys Phe Phe Thr Glu Pro Thr Ala Ser Pro Val
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5	gga gct ggc agc agc acg acg agg tac cgc ggc gta cgc cgg agg ccg	192
	Gly Ala Gly Ser Ser Thr Thr Arg Tyr Arg Gly Val Arg Arg Arg Pro	
	50 55 60	
10	tgg gga cga tac gcg gcg gag ata cgt gac cca atg tcg aag gag aga	240
	Trp Gly Arg Tyr Ala Ala Glu Ile Arg Asp Pro Met Ser Lys Glu Arg	
	65 70 75 80	
	cgt tgg ctc gga aca ttt gac acg gcg gaa caa gcc gct tgt gct tac	288
	Arg Trp Leu Gly Thr Phe Asp Thr Ala Glu Gln Ala Ala Cys Ala Tyr	
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	Asp Ser Ala Ala Arg Ala Phe Arg Gly Ala Lys Ala Arg Thr Asn Phe	
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20	act tat ccg aca gct gtc att atg cct gaa cca agg ttt tct ttt tcc	384
	Thr Tyr Pro Thr Ala Val Ile Met Pro Glu Pro Arg Phe Ser Phe Ser	
	115 120 125	
	aac aag aaa tct tcg ccg tct gct cgt tgt cct ctt cct tct cta ccg	432
	Asn Lys Lys Ser Ser Pro Ser Ala Arg Cys Pro Leu Pro Ser Leu Pro	
	130 135 140	
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	Leu Asp Ser Ser Thr Gln Asn Phe Tyr Gly Ala Pro Ala Ala Gln Arg	
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	Ile Tyr Asn Thr Gln Ser Ile Phe Leu Arg Asp Ala Ser Cys Ser Ser	
	165 170 175	
	cgt aaa acg act ccg tat aat aac tct ttc aac ggc tca tca tct tct	576
	Arg Lys Thr Thr Pro Tyr Asn Asn Ser Phe Asn Gly Ser Ser Ser Ser	
	180 185 190	
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	Tyr Ser Ala Ser Lys Thr Ala Cys Val Ser Tyr Ser Glu Asn Glu Asn	
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	Asn Glu Ser Phe Phe Pro Glu Glu Ser Ser Asp Thr Gly Leu Leu Gln	
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	Glu Val Val Gln Glu Phe Leu Lys Lys Asn Arg Gly Val Pro Pro Ser	
	225 230 235 240	
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	245 250 255	
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	260 265 270	
	gag act ttg tcg tcg aaa cta gat cgc tac ggg aat ttt caa gct aat	864
	Glu Thr Leu Ser Ser Lys Leu Asp Arg Tyr Gly Asn Phe Gln Ala Asn	
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Trp Gly Arg Tyr Ala Ala Glu Ile Arg Asp Pro Met Ser Lys Glu Arg
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Thr Tyr Pro Thr Ala Val Ile Met Pro Glu Pro Arg Phe Ser Phe Ser
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Asn Lys Lys Ser Ser Pro Ser Ala Arg Cys Pro Leu Pro Ser Leu Pro
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Ile Tyr Asn Thr Gln Ser Ile Phe Leu Arg Asp Ala Ser Cys Ser Ser
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Arg Lys Thr Thr Pro Tyr Asn Asn Ser Phe Asn Gly Ser Ser Ser Ser
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10 Glu Val Val Gln Glu Phe Leu Lys Lys Asn Arg Gly Val Pro Pro Ser
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260 265 270

20 Glu Thr Leu Ser Ser Lys Leu Asp Arg Tyr Gly Asn Phe Gln Ala Asn
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Asp Asp Gly Val Arg Ala Val Ala Asp Gly Gly Leu Ser Leu Gly Ser
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35 40 45

cca tgg gac atc caa gag tta tgt gga aga ggg aca gga gaa gag agg 192
Pro Trp Asp Ile Gln Glu Leu Cys Gly Arg Gly Thr Gly Glu Glu Arg
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		85 90 95	
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		Asp Lys Ala Ile Tyr Ser Lys Gln Glu Leu Val Gly Met Arg Lys Thr	
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		ctt gtc ttt tac aaa ggt agg gcc cca aat ggt cag aaa tct gat tgg	384
		Leu Val Phe Tyr Lys Gly Arg Ala Pro Asn Gly Gln Lys Ser Asp Trp	
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		Ile Met His Glu Tyr Arg Leu Glu Thr Asp Glu Asn Gly Pro Pro His	
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		Glu Ser Asn Trp Phe Thr Gln Gln Met Asp Val Gly Asn Gly Asn Tyr	
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		Tyr His Leu Pro Asp Leu Glu Ser Pro Arg Met Phe Gln Gly Ser Ser	
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		245 250 255	
		aac aca agt act ggt gat cat cat caa tca gga tta cta gtc aat gat	816
		Asn Thr Ser Thr Gly Asp His His Gln Ser Gly Leu Leu Val Asn Asp	
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		Ala Ser Gln Leu Ile Met Ser Gln Glu Glu Glu Val Asn Lys Asp	
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		Pro Ser Asp Asn Ser Ser Asn Glu Thr Phe His His Leu Ser Glu Glu	
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25 Pro Trp Asp Ile Gln Glu Leu Cys Gly Arg Gly Thr Gly Glu Glu Arg
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Arg Thr Asn Arg Ala Thr Gly Ser Gly Phe Trp Lys Ala Thr Gly Arg
85 90 95

35 Asp Lys Ala Ile Tyr Ser Lys Gln Glu Leu Val Gly Met Arg Lys Thr
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165 170 175

50 Glu Ser Asn Trp Phe Thr Gln Gln Met Asp Val Gly Asn Gly Asn Tyr
180 185 190

55 Tyr His Leu Pro Asp Leu Glu Ser Pro Arg Met Phe Gln Gly Ser Ser
195 200 205

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 245 250 255
 Asn Thr Ser Thr Gly Asp His His Gln Ser Gly Leu Leu Val Asn Asp
 15 260 265 270
 Asp His Asn Asp Gln Val Met Asp Trp Gln Thr Leu Asp Lys Phe Val
 275 280 285
 20 Ala Ser Gln Leu Ile Met Ser Gln Glu Glu Glu Glu Val Asn Lys Asp
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 Pro Ser Asp Asn Ser Ser Asn Glu Thr Phe His His Leu Ser Glu Glu
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	Lys Arg Ala Val Lys Ser Glu Ser Thr Val Ser Pro Val Val Ser Ala	
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	Tyr Asp Asn Ala Ala Ile Gln Leu Arg Gly Pro Asp Ala Leu Thr Asn	
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35	ttc tca gtc act ccg aca aca gcg acg gag aag aaa gcc cca cca ccg	576
	Phe Ser Val Thr Pro Thr Thr Ala Thr Glu Lys Lys Ala Pro Pro Pro	
	180 185 190	
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	Ser Pro Val Lys Lys Lys Lys Lys Lys Asn Asn Lys Ser Lys Lys Ser	
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	115 120 125	
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Leu Arg Ser Lys Ala Ser Pro Val Val Gly Arg Ser Ser Thr Thr Val
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Ser Lys Pro Val Gly Val Arg Gln Arg Lys Trp Gly Lys Trp Ala Ala
130 135 140

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145 150 155 160

gag acg ctt gaa caa gca gct gat gct tat gct acc aag aag ctt gag 528
Glu Thr Leu Glu Gln Ala Ala Asp Ala Tyr Ala Thr Lys Lys Leu Glu
165 170 175

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Phe Asp Ala Leu Ala Ala Ala Thr Ser Ala Ala Ser Ser Val Leu Ser
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	Asn Glu Ser Gly Ser Met Ile Ser Ala Ser Gly Ser Ile Asp Leu	
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	gat ttt ctc tta aca gaa gag aac aac aac caa atg ttg gat gat tac	816
	Asp Phe Leu Leu Thr Glu Glu Asn Asn Asn Gln Met Leu Asp Asp Tyr	
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	Cys Gly Ile Asp Asp Leu Asp Ile Ile Gly Leu Glu Cys Asp Gly Pro	
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	Leu Ile Gly Thr Thr Ile Asp Lys Tyr Ala Phe Val Asp His Ile Ala	
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	Asp Pro Tyr Ala Thr Asp Tyr Ser Ser Ser Glu Glu Glu Glu Arg Ser	
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Gly His Gly Cys Trp Ser Ser Val Pro Lys Leu Ala Gly Leu Gln Arg
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85 90 95

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115 120 125

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130 135 140

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195 200 205

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 Asp Ser Met His Arg Val Ile Pro Val Leu Asp Val Phe Glu Val Glu
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 Glu Gln Trp Phe Phe Val Pro Arg Gln Arg Glu Ala Arg Gly
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 Gly Arg Pro Ser Arg Thr Thr Gly Ser Gly Tyr Trp Lys Ala Thr Gly
 85 90 95

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 Ser Pro Gly Pro Val Phe Ser Lys Asp Asn Lys Met Ile Gly Ala Lys
 100 105 110

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	tat cca acg gct gag atg gca gct cga gct cat gac gta gcg gct tta Tyr Pro Thr Ala Glu Met Ala Ala Arg Ala His Asp Val Ala Ala Leu 130 135 140						432
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40	tcg tcc acg tgg cag ctt tgt gga gcc gat gct ggg ttt cgg ctt gaa Ser Ser Thr Trp Gln Leu Cys Gly Ala Asp Ala Gly Phe Arg Leu Glu 275 280 285	864
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 Val Leu Asn Pro Gln Val Phe Gln Gln Met Gly Lys Glu Gln Phe Arg
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Phe Arg Asn Pro Arg Ile Val Arg Val Ser Arg Thr Phe Gly Gly Lys
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Arg Leu Gly Leu Ser Gln Pro Ser Lys Val Ile Asp Trp Leu Leu Glu
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Ala Ala Lys Asp Asp Val Asp Lys Leu Pro Pro Leu Gln Phe Pro His
115 120 125
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	Thr His Gly Glu Gly Lys Trp Ser Thr Leu Pro Asn Gln Ala Gly Leu	
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	Arg Leu Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn His Trp Asn Ser	
	100 105 110	
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	Phe	Leu	Arg	Ser	Glu	Asp	Val	Tyr	Cys	Lys	Asn	Pro	Trp	Thr	Leu	Phe	
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	Trp	Arg	Ile	Ile	Gly	Arg	Asp	Arg	Val	Ile	Lys	Ser	Val	Thr	Thr	Gly	
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 Asn Glu His Arg Ile Lys Ile Ser Ser Gly Asp Gly Tyr Trp Lys Pro
 85 90 95

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 Ile Gly Lys Glu Arg Pro Ile Ile Ala Cys Gly Lys Thr Phe Gly Ile
 100 105 110

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 Arg Arg Thr Leu Ala Phe Tyr Glu Thr Asn Lys Ser Ser Ser Tyr Cys
 115 120 125

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 130 135 140

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 50 55 60

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 Ser Ala Lys Val Ser Gly Glu Trp Ala Val Tyr Asn Val Tyr Glu Arg
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	Ala Ala Glu Ala Leu Ala Asp Leu Ala Gln Leu Ala Ile Met Arg Glu	
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	Gln Val Phe Glu Ser Ala Ala Ser Trp Gly Ser Lys Gly Lys Arg Val	
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	Pro Pro Asp Ser Asp Thr Leu Pro Thr Pro Asp Leu Ala Glu Glu Arg	
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	Leu Val Lys Glu Glu Glu Glu Glu Glu Glu Val Glu Pro Ile Thr Lys	
	100 105 110	
40	gaa cta act aaa gct ccg gtt aaa tct gaa atc aat ggt gaa aca cct	384
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	115 120 125	
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	145 150 155 160	
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	Arg Arg Arg Gln Ala Met Cys Glu Glu Leu Ser Lys Lys Ala Ala Asp	
	180 185 190	
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	Leu Thr Tyr Glu Asn Glu Asn Leu Arg Arg Glu Lys Asp Trp Ala Leu	
	195 200 205	
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20	gct tct gct aaa act att acc acg cag gag cat gaa aat gct gca gat Ala Ser Ala Lys Thr Ile Thr Thr Gln Glu His Glu Asn Ala Ala Asp 290 295 300	912
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25	Arg	Lys	Arg	Val	Lys	Thr	Glu	Ser	Pro	Pro	Ser	Asp	Ser	Leu	Leu	Lys	65	70	75	
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70	Lys	Glu	Phe	Gln	Ser	Leu	Glu	Thr	Ile	Asn	Lys	His	Leu	Lys	Glu	Gln	210	215	220	
75	Val	Leu	Lys	Ser	Val	Lys	Pro	Asp	Thr	Lys	Glu	Pro	Glu	Glu	Ser	Pro	225	230	235	
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	Leu Pro Pro Pro Asp His Ser Asn Gly Val Pro Phe Gly Leu Gln Asp 325 330 335		
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25	Ala Arg Pro Met Asp Val Thr Glu Thr Pro Arg Ser His Leu Pro Thr 355 360 365		
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	Phe Ser Pro Val Gln Glu Lys Ile Thr Glu Arg Pro Val Cys Ser Asn	
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Ile Thr Arg His Leu Leu Gly Gly Gly Glu Asn Glu Leu Arg Leu
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aat gag tca aca ccg agt tcg tgt ttc aca gag agt tgg gga ggt ttg      144
Asn Glu Ser Thr Pro Ser Ser Cys Phe Thr Glu Ser Trp Gly Gly Leu
35      40      45

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Pro Leu Lys Glu Asn Asp Ser Glu Asp Met Leu Val Tyr Gly Leu Leu
25      50      55      60

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30      85      90      95

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Met Glu Glu Lys Pro Lys Lys Ala Ile Pro Val Thr Glu Thr Ala Val
35      100      105      110

aag gcg aag cat tac aga gga gtg agg cag aga ccg tgg ggg aaa ttc      384
Lys Ala Lys His Tyr Arg Gly Val Arg Gln Arg Pro Trp Gly Lys Phe
40      115      120      125

gcg gcg gag ata cgt gat ccg gcg aag aat gga gct agg gtt tgg tta      432
Ala Ala Glu Ile Arg Asp Pro Ala Lys Asn Gly Ala Arg Val Trp Leu
130      135      140

ggg acg ttt gag acg gcg gaa gat gcg gct tta gct tac gat ata gct      480
Gly Thr Phe Glu Thr Ala Glu Asp Ala Ala Leu Ala Tyr Asp Ile Ala
45      145      150      155      160

gct ttt agg atg cgt ggt tcc cgc gct tta ttg aat ttt ccg ttg agg      528
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50      180      185      190

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Phe Asp Phe Pro Ala Val Lys Val Glu Pro Thr Glu Asn Phe Thr Ala
 85 90 95

Met Glu Glu Lys Pro Lys Lys Ala Ile Pro Val Thr Glu Thr Ala Val
 100 105 110

Lys Ala Lys His Tyr Arg Gly Val Arg Gln Arg Pro Trp Gly Lys Phe
 115 120 125

Ala Ala Glu Ile Arg Asp Pro Ala Lys Asn Gly Ala Arg Val Trp Leu
 130 135 140

Gly Thr Phe Glu Thr Ala Glu Asp Ala Ala Leu Ala Tyr Asp Ile Ala
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Ala Phe Arg Met Arg Gly Ser Arg Ala Leu Leu Asn Phe Pro Leu Arg
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Val Asn Ser Gly Glu Pro Asp Pro Val Arg Ile Thr Ser Lys Arg Ser

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	Glu Pro Cys Cys Lys Ile Lys Met Lys Lys Arg Asp Ile Thr Pro Ile						
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15 Asp Asn Ser Ile Ile Tyr Asn Lys Asp Lys Lys Lys Asp Gln Leu Val
 180 185 190

20 Asn Asn Leu Ile Asp Gly Asp Asn Met Trp Leu Glu Lys Phe Leu Glu
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Glu Ser Gln Glu Val Asp Ile Leu Val Pro Glu Ala Thr Thr Thr Glu
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 Trp Ser Pro Glu Glu Asp Glu Lys Leu Ile Arg Tyr Ile Thr Thr His
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55 ggc tat gga tgt tgg agt gaa gtc cct gaa aaa gca ggg ctt caa aga 144
 Gly Tyr Gly Cys Trp Ser Glu Val Pro Glu Lys Ala Gly Leu Gln Arg
 35 40 45

60 tgt gga aaa agt tgt aga ttg cga tgg ata aac tat ctt cga cct gat 192
 Cys Gly Lys Ser Cys Arg Leu Arg Trp Ile Asn Tyr Leu Arg Pro Asp
 50 55 60

65 atc agg aga gga agg ttc tct cca gaa gaa gag aaa ttg atc ata agc 240
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		85		90		95			
	ccg gga aga aca gat aac gag att aaa aac tat tgg aat tca tgg att Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Ser Trp Ile	336							
		100		105		110			
10	aag aaa aag ata cga aaa ccg cac cat cat tac agt cgt cat caa ccg Lys Lys Lys Ile Arg Lys Pro His His His Tyr Ser Arg His Gln Pro	384							
		115		120		125			
15	tca gta act act gtg aca ttg aat gcg gac act aca tcg att gcc act Ser Val Thr Thr Val Thr Leu Asn Ala Asp Thr Thr Ser Ile Ala Thr	432							
		130		135		140			
	acc atc gag gcc tct acc acc aca aca tcg act atc gat aac tta cat Thr Ile Glu Ala Ser Thr Thr Thr Thr Ser Thr Ile Asp Asn Leu His	480							
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		165		170		175			
25	caa gaa act aat ata aag att caa gaa act ttt ttc tcc cat aaa cct Gln Glu Thr Asn Ile Lys Ile Gln Glu Thr Phe Phe Ser His Lys Pro	576							
		180		185		190			
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		195		200		205			
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		260		265		270			
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		290		295		300			
50	gaa atg gcg tcc aca ttc gaa tgt tta aag agg caa gaa cta agc tat Glu Met Ala Ser Thr Phe Glu Cys Leu Lys Arg Gln Glu Leu Ser Tyr	960							
		305		310		315		320	
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		325		330		335			

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 cca tca atg aat ttg gga tca tct gcc tta tct tct tct ttc cct tct 1104
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 Ile Arg Arg Gly Arg Phe Ser Pro Glu Glu Glu Lys Leu Ile Ile Ser
 65 70 75 80

35 Leu His Gly Val Val Gly Asn Arg Trp Ala His Ile Ala Ser His Leu
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40 Pro Gly Arg Thr Asp Asn Glu Ile Lys Asn Tyr Trp Asn Ser Trp Ile
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 Lys Lys Lys Ile Arg Lys Pro His His His Tyr Ser Arg His Gln Pro
 115 120 125

45 Ser Val Thr Thr Val Thr Leu Asn Ala Asp Thr Thr Ser Ile Ala Thr
 130 135 140

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 Phe Asp Gly Phe Thr Asp Ser Pro Asn Gln Leu Asn Phe Thr Asn Asp
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 195 200 205
 10 Ser Glu Asn Ile Ile Thr Asn Asn Asn Lys Asn Asn Asp His Asp Asp
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 Thr Gln Arg Gly Gly Arg Glu Asn Val Cys Glu Gln Ala Phe Leu Thr
 225 230 235 240
 15 Thr Asn Thr Glu Glu Trp Asp Met Asn Leu Arg Gln Gln Glu Pro Phe
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 20 Gln Val Pro Thr Leu Ala Ser His Val Phe Asn Asn Ser Ser Asn Ser
 260 265 270
 Asn Ile Asp Thr Val Ile Ser Tyr Asn Leu Pro Ala Leu Ile Glu Gly
 275 280 285
 25 Asn Val Asp Asn Ile Val His Asn Glu Asn Ser Asn Val Gln Asp Gly
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	His Tyr Leu Lys Arg Lys Ala Ser Ala Pro Leu Pro Val Ala Ile							
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	Ile Ala Glu Val Asp Leu Tyr Lys Phe Asp Pro Trp Glu Leu Pro Ala							
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	cgg aag tat cca aac gga gca aga cca aac aga gcg gcg act tca ggt							288
	Arg Lys Tyr Pro Asn Gly Ala Arg Pro Asn Arg Ala Ala Thr Ser Gly							
			85		90		95	
20	tat tgg aaa gcg acc ggt aca gat aaa ccg gta ctt gct tcc gac ggt							336
	Tyr Trp Lys Ala Thr Gly Thr Asp Lys Pro Val Leu Ala Ser Asp Gly							
			100		105		110	
25	aac caa aag gtg ggc gtg aag aag gca cta gtc ttc tac agt ggt aaa							384
	Asn Gln Lys Val Gly Val Lys Lys Ala Leu Val Phe Tyr Ser Gly Lys							
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	cca cca aaa ggc gtt aaa agt gat tgg atc atg cat gag tat cgt ctc							432
	Pro Pro Lys Gly Val Lys Ser Asp Trp Ile Met His Glu Tyr Arg Leu							
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	Ile Glu Asn Lys Pro Asn Asn Arg Pro Pro Gly Cys Asp Phe Gly Asn							
			145		150		155	160
35	aaa aaa aac tca ctc aga ctt gat gat tgg gtg tta tgt aga atc tac							528
	Lys Lys Asn Ser Leu Arg Leu Asp Asp Trp Val Leu Cys Arg Ile Tyr							
			165		170		175	
	aag aag aac aac gca agt cga cat gtt gat aac gat aag gat cat gat							576
	Lys Lys Asn Asn Ala Ser Arg His Val Asp Asn Asp Lys Asp His Asp							
			180		185		190	
40	atg atc gat tac att ttc agg aag att cct ccg tct tta tca atg gcg							624
	Met Ile Asp Tyr Ile Phe Arg Lys Ile Pro Pro Ser Leu Ser Met Ala							
			195		200		205	
45	gct gct tct aca gga ctt cac caa cat cat cat aat gtc tca aga tca							672
	Ala Ala Ser Thr Gly Leu His Gln His His Asn Val Ser Arg Ser							
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	atg aat ttc ttc cct ggc aaa ttc tcc ggt ggt ggt tac ggg att ttc							720
	Met Asn Phe Phe Pro Gly Lys Phe Ser Gly Gly Gly Tyr Gly Ile Phe							
			225		230		235	240
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	Ser Asp Gly Gly Asn Thr Ser Ile Tyr Asp Gly Gly Gly Met Ile Asn							
			245		250		255	
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	Asn Ile Gly Thr Asp Ser Val Asp His Asp Asn Asn Ala Asp Val Val							
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275 280 285

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Lys Arg Thr Leu Pro Val Pro Tyr Trp Pro Val Ala Asp Glu Glu Gln
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10 gat gca tct ccg agc aaa cgg ttt cac ggt gta gga gga gga gga gga 960
Asp Ala Ser Pro Ser Lys Arg Phe His Gly Val Gly Gly Gly Gly Gly
305 310 315 320

15 gat tgt tcg aac atg tct tcc tcc atg atg gaa gag act cca cca ttg 1008
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325 330 335

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Lys Ala Ser Phe Gly Glu Gln Glu Trp Tyr Phe Phe Ser Pro Arg Asp
65 70 75 80

45 Arg Lys Tyr Pro Asn Gly Ala Arg Pro Asn Arg Ala Ala Thr Ser Gly
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50 Tyr Trp Lys Ala Thr Gly Thr Asp Lys Pro Val Leu Ala Ser Asp Gly
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Asn Gln Lys Val Gly Val Lys Lys Ala Leu Val Phe Tyr Ser Gly Lys
115 120 125

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 180 185 190
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 Met Ile Asp Tyr Ile Phe Arg Lys Ile Pro Pro Ser Leu Ser Met Ala
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 Gly Leu Asn His Ala Ser Ser Ser Gly Pro Met Met Met Ala Asn Leu
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	Leu Arg Ala Val Asp Leu Thr Ile Ile Asn Gly Val Arg Asn Val Glu	
	20 25 30	
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	Thr Ser Arg Pro Phe Gln Val Asn Pro Thr Val Ser Leu Glu Pro Lys	
	35 40 45	
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	Ser Thr Gly Pro Pro Leu Lys Arg Ala Ser Thr Lys Asp Arg His Thr	
	65 70 75 80	
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	Lys Val Glu Gly Arg Gly Arg Arg Ile Arg Met Pro Ala Thr Cys Ala	
	85 90 95	
35	gct agg att ttt caa tta act cga gag tta ggt cac aaa tcc gac ggc	336
	Ala Arg Ile Phe Gln Leu Thr Arg Glu Leu Gly His Lys Ser Asp Gly	
	100 105 110	
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	115 120 125	
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	Thr Thr Thr Thr Ile Gln Pro Pro Gln Ala Leu Ala Ser Ser Thr Val	
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	cat atg cca tat ggc ctc aat gca tct gct tac gct ctc aac aac cct His Met Pro Tyr Gly Leu Asn Ala Ser Ala Tyr Ala Leu Asn Asn Pro 210 215 220			672
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	Ser	His	Lys	Gly	Lys	Leu	Phe	Glu	Tyr	Ser	Thr	Asp	Ser	Cys	Met	Glu	
55		50					55					60					

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	Lys	Ile	Leu	Glu	Arg	Tyr	Glu	Arg	Tyr	Ser	Tyr	Ala	Glu	Arg	Gln	Leu	
	65					70				75					80		
5	Ile	Ala	Pro	Glu	Ser	Asp	Val	Asn	Thr	Asn	Trp	Ser	Met	Glu	Tyr	Asn	
					85					90					95		
	Arg	Leu	Lys	Ala	Lys	Ile	Glu	Leu	Leu	Glu	Arg	Asn	Gln	Arg	His	Tyr	
10				100					105					110			
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			115					120					125				
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			130				135					140					
	Gln	Leu	Met	Tyr	Glu	Ser	Ile	Asn	Glu	Leu	Gln	Lys	Lys	Glu	Lys	Ala	
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					165					170					175		
25	Lys	Ile	Leu	Arg	Ala	Gln	Gln	Glu	Gln	Trp	Asp	Gln	Gln	Asn	Gln	Gly	
				180					185					190			
	His	Asn	Met	Pro	Pro	Pro	Leu	Pro	Pro	Gln	Gln	His	Gln	Ile	Gln	His	
30			195					200					205				
	Pro	Tyr	Met	Leu	Ser	His	Gln	Pro	Ser	Pro	Phe	Leu	Asn	Met	Gly	Gly	
			210				215					220					
35	Leu	Tyr	Gln	Glu	Asp	Asp	Pro	Met	Ala	Met	Arg	Arg	Asn	Asp	Leu	Glu	
						230					235					240	
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	Ala	Pro	Gly	Thr	Ala	Ser	Val	Ala	Thr	Ser	Leu	Ala	Pro	Gly	Phe	Arg	

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10	cca tta cct ctt ctg caa tac aaa cgt aga cgc caa agc gag tcc aac Pro Leu Pro Leu Leu Gln Tyr Lys Arg Arg Arg Gln Ser Glu Ser Asn 305 310 315 320	960
15	aac aac tca agc agg aac aca cag gac cat tgt tcg tcc aca aca aca Asn Asn Ser Ser Arg Asn Thr Gln Asp His Cys Ser Ser Thr Thr Thr 325 330 335	1008
20	act gtc gac aat aca acc act tta atc tca tca tct gcc gct gcc acc Thr Val Asp Asn Thr Thr Thr Leu Ile Ser Ser Ser Ala Ala Ala Thr 340 345 350	1056
25	aac act gcc atc tct gca ttg ctt gag ttc tca ctc atg ggt atc tcc Asn Thr Ala Ile Ser Ala Leu Leu Glu Phe Ser Leu Met Gly Ile Ser 355 360 365	1104
30	gac aag aaa gaa aag ccg cag caa ccg cta cgt cct cac aag gaa cct Asp Lys Lys Glu Lys Pro Gln Gln Pro Leu Arg Pro His Lys Glu Pro 370 375 380	1152
35	ttg cct cct caa act cca ctt gca tct cct gaa gag aag gtt aat gat Leu Pro Pro Gln Thr Pro Leu Ala Ser Pro Glu Glu Lys Val Asn Asp 385 390 395 400	1200
40	ctc cag aag gag att cac cag atg tct gtt gaa aga gaa act ttc aag Leu Gln Lys Glu Ile His Gln Met Ser Val Glu Arg Glu Thr Phe Lys 405 410 415	1248
45	ctt gaa atg atg agt gca gaa gct atg atc agt att ctc cag tca agg Leu Glu Met Met Ser Ala Glu Ala Met Ile Ser Ile Leu Gln Ser Arg 420 425 430	1296
50	atc gat gcg ctg cgt cag gag aac gag gaa ctc aag aag aac aat gct Ile Asp Ala Leu Arg Gln Glu Asn Glu Glu Leu Lys Lys Asn Asn Ala 435 440 445	1344
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5	Tyr	Lys	His	Glu	Pro	Trp	Asp	Leu	Ala	Val	Phe	Ser	Arg	Leu	Lys	Thr	
	65					70					75					80	
	Arg	Asp	Gln	Glu	Trp	Tyr	Phe	Tyr	Ser	Ala	Leu	Asp	Lys	Lys	Tyr	Gly	
10					85					90					95		
	Asn	Gly	Ala	Arg	Met	Asn	Arg	Ala	Thr	Asn	Arg	Gly	Tyr	Trp	Lys	Ala	
					100				105						110		
15	Thr	Gly	Lys	Asp	Arg	Glu	Ile	Arg	Arg	Asp	Ile	Leu	Leu	Leu	Gly	Met	
			115					120					125				
	Lys	Lys	Thr	Leu	Val	Phe	His	Ser	Gly	Arg	Ala	Pro	Asp	Gly	Leu	Arg	
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	Thr	Asn	Trp	Val	Met	His	Glu	Tyr	Arg	Leu	Val	Glu	Tyr	Glu	Thr	Glu	
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25	Lys	Asn	Gly	Asn	Leu	Val	Gln	Asp	Ala	Tyr	Val	Leu	Cys	Arg	Val	Phe	
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	Met	Glu	Glu	Glu	Trp	Ala	Asp	Asp	Glu	Gly	Ala	Leu	Ile	Pro	Gly	Ile	
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		210					215					220					
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					245					250					255		
45	Gln	Asn	His	His	Glu	Asn	Asp	Leu	Lys	Pro	Glu	Glu	His	Asn	Asn	Asn	
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50			275					280					285				
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		290					295					300					
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	305					310						315					320
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	Thr	Val	Asp	Asn	Thr	Thr	Thr	Leu	Ile	Ser	Ser	Ser	Ala	Ala	Ala	Thr	
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10	Asn	Thr	Ala	Ile	Ser	Ala	Leu	Leu	Glu	Phe	Ser	Leu	Met	Gly	Ile	Ser	
			355					360					365				
15	Asp	Lys	Lys	Glu	Lys	Pro	Gln	Gln	Pro	Leu	Arg	Pro	His	Lys	Glu	Pro	
		370					375					380					
	Leu	Pro	Pro	Gln	Thr	Pro	Leu	Ala	Ser	Pro	Glu	Glu	Lys	Val	Asn	Asp	
	385					390					395					400	
20	Leu	Gln	Lys	Glu	Ile	His	Gln	Met	Ser	Val	Glu	Arg	Glu	Thr	Phe	Lys	
					405					410					415		
25	Leu	Glu	Met	Met	Ser	Ala	Glu	Ala	Met	Ile	Ser	Ile	Leu	Gln	Ser	Arg	
				420					425					430			
	Ile	Asp	Ala	Leu	Arg	Gln	Glu	Asn	Glu	Glu	Leu	Lys	Lys	Asn	Asn	Ala	
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	1				5					10					15		
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	Leu	Leu	Thr	Asp	Phe	Val	Ser	Met	Glu	Thr	Asp	His	Pro	Ser	Leu	Phe	
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	Thr	Asn	Gln	Leu	His	Asn	Phe	His	Ser	Glu	Thr	Gly	Pro	Arg	Thr	Ile	
			35					40					45				
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55	Thr	Asn	Gln	Ser	Pro	Lys	Pro	Asn	Ser	Thr	Leu	Asn	Gln	Arg	Lys	Pro	
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10	aaa gag gaa gaa gag agg cac tac agg gga gtg aga cga aga ccg tgg Lys Glu Glu Glu Glu Arg His Tyr Arg Gly Val Arg Arg Arg Pro Trp 85 90 95	288
15	gga aaa tac gcg gcg gag att agg gat ccg aac aaa aag ggt tgt agg Gly Lys Tyr Ala Ala Glu Ile Arg Asp Pro Asn Lys Lys Gly Cys Arg 100 105 110	336
20	atc tgg ctt ggg act tac gac act gcc gtg gaa gct gga aga gct tat Ile Trp Leu Gly Thr Tyr Asp Thr Ala Val Glu Ala Gly Arg Ala Tyr 115 120 125	384
25	gac caa gcg gcg ttt caa tta cgt gga aga aaa gca atc ttg aat ttc Asp Gln Ala Ala Phe Gln Leu Arg Gly Arg Lys Ala Ile Leu Asn Phe 130 135 140	432
30	cct ctc gat gtt agg gtt acg tca gaa act tgt tct ggg gaa gga gtt Pro Leu Asp Val Arg Val Thr Ser Glu Thr Cys Ser Gly Glu Gly Val 145 150 155 160	480
35	atc gga tta ggg aaa cga aag cga gat aag ggt tct ccg ccg gaa gag Ile Gly Leu Gly Lys Arg Lys Arg Asp Lys Gly Ser Pro Pro Glu Glu 165 170 175	528
40	gag aag gcg gct agg gtt aaa gtg gag gaa gaa gag agt aat acg tcg Glu Lys Ala Ala Arg Val Lys Val Glu Glu Glu Glu Ser Asn Thr Ser 180 185 190	576
45	gag acg acg gag gct gag gtt gag ccg gtg gta cca ttg acg ccg tca Glu Thr Thr Glu Ala Glu Val Glu Pro Val Val Pro Leu Thr Pro Ser 195 200 205	624
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10	Pro	Leu	Pro	Asn	Leu	Ser	Val	Ser	Arg	Thr	Val	Ser	Thr	Lys	Thr	Glu
	65					70					75					80
	Lys	Glu	Glu	Glu	Glu	Arg	His	Tyr	Arg	Gly	Val	Arg	Arg	Arg	Pro	Trp
					85					90					95	
15	Gly	Lys	Tyr	Ala	Ala	Glu	Ile	Arg	Asp	Pro	Asn	Lys	Lys	Gly	Cys	Arg
				100					105					110		
20	Ile	Trp	Leu	Gly	Thr	Tyr	Asp	Thr	Ala	Val	Glu	Ala	Gly	Arg	Ala	Tyr
			115					120					125			
	Asp	Gln	Ala	Ala	Phe	Gln	Leu	Arg	Gly	Arg	Lys	Ala	Ile	Leu	Asn	Phe
		130					135					140				
25	Pro	Leu	Asp	Val	Arg	Val	Thr	Ser	Glu	Thr	Cys	Ser	Gly	Glu	Gly	Val
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30	Ile	Gly	Leu	Gly	Lys	Arg	Lys	Arg	Asp	Lys	Gly	Ser	Pro	Pro	Glu	Glu
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	Glu	Lys	Ala	Ala	Arg	Val	Lys	Val	Glu	Glu	Glu	Glu	Ser	Asn	Thr	Ser
				180					185					190		
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			195					200					205			
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[illegible]

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	Cys Glu Ser Ser Met Ser Leu Glu Arg Pro Lys Lys Tyr Arg Gly Val 50 55 60			
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15	Lys Ala Thr Arg Val Trp Leu Gly Thr Phe Glu Thr Ala Glu Ala Ala 85 90 95			
	Ala Arg Ala Tyr Asp Ala Ala Ala Leu Arg Phe Arg Gly Ser Lys Ala 100 105 110			
20	Lys Leu Asn Phe Pro Glu Asn Val Gly Thr Gln Thr Ile Gln Arg Asn 115 120 125			
25	Ser His Phe Leu Gln Asn Ser Met Gln Pro Ser Leu Thr Tyr Ile Asp 130 135 140			
	Gln Cys Pro Thr Leu Leu Ser Tyr Ser Arg Cys Met Glu Gln Gln Gln 145 150 155 160			
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5	aaa tcc gat cca tgg gat tta cca gct aag gct cca ttt ggg gag aaa Lys Ser Asp Pro Trp Asp Leu Pro Ala Lys Ala Pro Phe Gly Glu Lys 50 55 60	192
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15	aga cca aac aga gca gct gcg tct gga tat tgg aaa gca acc gga aca Arg Pro Asn Arg Ala Ala Ala Ser Gly Tyr Trp Lys Ala Thr Gly Thr 85 90 95	288
20	gat aaa ttg att gcg gta cca aat ggt gaa ggg ttt cat gaa aac att Asp Lys Leu Ile Ala Val Pro Asn Gly Glu Gly Phe His Glu Asn Ile 100 105 110	336
25	ggt ata aaa aaa gct ctt gtg ttt tat aga gga aag cct cca aaa ggt Gly Ile Lys Lys Ala Leu Val Phe Tyr Arg Gly Lys Pro Pro Lys Gly 115 120 125	384
30	gtt aaa acc aat tgg atc atg cat gaa tat cgt ctt gcc gat tca tta Val Lys Thr Asn Trp Ile Met His Glu Tyr Arg Leu Ala Asp Ser Leu 130 135 140	432
35	tct ccc aaa aga att aac tct tct agg agc ggt ggt agc gaa gtt aat Ser Pro Lys Arg Ile Asn Ser Ser Arg Ser Gly Gly Ser Glu Val Asn 145 150 155 160	480
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45	gat tgg gtt ctt tgc cgg att tac aag aaa tca cac gct tca ttg tca Asp Trp Val Leu Cys Arg Ile Tyr Lys Lys Ser His Ala Ser Leu Ser 180 185 190	576
50	tca cct gat gtt gct ttg gtc aca agc aat caa gag cat gag gaa aat Ser Pro Asp Val Ala Leu Val Thr Ser Asn Gln Glu His Glu Glu Asn 195 200 205	624
55	gac aac gaa cca ttc gta gac cgc gga acc ttt ttg cca aat ttg caa Asp Asn Glu Pro Phe Val Asp Arg Gly Thr Phe Leu Pro Asn Leu Gln 210 215 220	672
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5 atg atg aac aca tat agt tac aat aat agt ata gat caa atg gat cat 1008
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 325 330 335

10 agt atg atg caa caa cct agt ttc ctg aac cag gaa ctc atg atg agt 1056
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 35 40 45

30 Lys Ser Asp Pro Trp Asp Leu Pro Ala Lys Ala Pro Phe Gly Glu Lys
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35 Glu Trp Tyr Phe Phe Ser Pro Arg Asp Arg Lys Tyr Pro Asn Gly Ala
 65 70 75 80

Arg Pro Asn Arg Ala Ala Ala Ser Gly Tyr Trp Lys Ala Thr Gly Thr
 85 90 95

40 Asp Lys Leu Ile Ala Val Pro Asn Gly Glu Gly Phe His Glu Asn Ile
 100 105 110

45 Gly Ile Lys Lys Ala Leu Val Phe Tyr Arg Gly Lys Pro Pro Lys Gly
 115 120 125

Val Lys Thr Asn Trp Ile Met His Glu Tyr Arg Leu Ala Asp Ser Leu
 130 135 140

50 Ser Pro Lys Arg Ile Asn Ser Ser Arg Ser Gly Gly Ser Glu Val Asn
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55 Asn Asn Phe Gly Asp Arg Asn Ser Lys Glu Tyr Ser Met Arg Leu Asp

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	tat ctc cgg aag aaa gtt aat agc atc gag atc gat ctt gat gtc att Tyr Leu Arg Lys Lys Val Asn Ser Ile Glu Ile Asp Leu Asp Val Ile 35 40 45	144
10	cgc gac gtt gat ctc aac aag ctc gag cct tgg gac att caa gag atg Arg Asp Val Asp Leu Asn Lys Leu Glu Pro Trp Asp Ile Gln Glu Met 50 55 60	192
15	tgt aaa ata gga aca acg cca caa aac gac tgg tat ttc ttt agc cac Cys Lys Ile Gly Thr Thr Pro Gln Asn Asp Trp Tyr Phe Phe Ser His 65 70 75 80	240
	aag gac aaa aaa tat ccg acg gga acg aga act aac aga gcc act gcg Lys Asp Lys Lys Tyr Pro Thr Gly Thr Arg Thr Asn Arg Ala Thr Ala 85 90 95	288
20	gct gga ttt tgg aaa gca act ggc cgc gac aag atc ata tat agc aat Ala Gly Phe Trp Lys Ala Thr Gly Arg Asp Lys Ile Ile Tyr Ser Asn 100 105 110	336
25	ggc cgt aga att ggg atg aga aag act ctt gtt ttc tac aaa ggc cga Gly Arg Arg Ile Gly Met Arg Lys Thr Leu Val Phe Tyr Lys Gly Arg 115 120 125	384
	gct cct cac ggc caa aaa tct gat tgg atc atg cat gaa tat aga ctc Ala Pro His Gly Gln Lys Ser Asp Trp Ile Met His Glu Tyr Arg Leu 130 135 140	432
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40	gga gct tcc ctg agc ggc ggc gga gat acg ccg aag acg aca tca tct Gly Ala Ser Leu Ser Gly Gly Gly Asp Thr Pro Lys Thr Thr Ser Ser 195 200 205	624
45	cag atc ttc aac gag gat act ctc gac caa ttt ctt gaa ctt atg ggg Gln Ile Phe Asn Glu Asp Thr Leu Asp Gln Phe Leu Glu Leu Met Gly 210 215 220	672
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	Leu Asn Gly Pro Thr Ser Tyr Ser Ile Thr Ala Val Asn Glu Ser His	
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	Thr Pro Glu Met Glu Leu Trp Asn Thr Thr Thr Ser Ser Leu Ser Ser	
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15 act cac ggc gaa ggc aaa tgg agc act ctc cct aac caa gct ggt ctc 144
 Thr His Gly Glu Gly Lys Trp Ser Thr Leu Pro Asn Gln Ala Gly Leu
 35 40 45

aag agg tgt ggc aaa agc tgt aga ctt cgg tgg aag aac tac cta aga 192
 Lys Arg Cys Gly Lys Ser Cys Arg Leu Arg Trp Lys Asn Tyr Leu Arg
 50 55 60

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 85 90 95

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 100 105 110

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 115 120 125

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 145 150 155 160

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 180 185 190

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gga gag ttg gtt cct gct caa ggt aat ctc gat ctc aat aga cct ttc 720
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 225 230 235 240

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	acc aaa gtt tcg aat atg ttt ggt gga aca cga gag aaa tgc gtt ggt Thr Lys Val Ser Asn Met Phe Gly Gly Thr Arg Glu Lys Cys Val Gly 100 105 110			336
	tgc gac aaa acc gtg tat cca att gag aag gta tcg gtg aat gga aca Cys Asp Lys Thr Val Tyr Pro Ile Glu Lys Val Ser Val Asn Gly Thr 115 120 125			384
	ttg tac cac aag agc tgc ttc aag tgt aca cat gga ggc tgc acg ata Leu Tyr His Lys Ser Cys Phe Lys Cys Thr His Gly Gly Cys Thr Ile 130 135 140			432

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Cys Asp Lys Thr Val Tyr Pro Ile Glu Lys Val Ser Val Asn Gly Thr
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His His Ile Gln Leu Ile Lys Glu Lys Gly Asn Leu Ser Gln Leu Glu
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50 Claims

1. A plant, in which a chimeric protein obtained by fusing a transcription factor consisting of any one of the following proteins (a) to (c) and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor is expressed, into which a gene encoding a transcription factor consisting of any of the following proteins (d) to (f) is introduced, or in which an expression control region of an endogenous gene corresponding to the gene is modified:

(a) a protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76;

(b) a protein having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76;

(c) a protein having transactivation activity encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence shown in any of the odd-numbered SEQ ID NOS: 1 to 76;

(d) a protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 77 to 84;

(e) a protein having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to the amino acid sequence shown in any of the even-numbered SEQ ID NOS: 77 to 84; and

(f) a protein having transactivation activity encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence shown in any of the odd-numbered SEQ ID NOS: 77 to 84.

2. The plant according to claim 1, wherein transactivation activity of the transcription factor is repressed in the chimeric protein.

3. The plant according to claim 1, wherein the chimeric protein has transcriptional repressor activity.

4. The plant according to claim 1, wherein the functional peptide has an amino acid sequence expressed by any one of the following formulae (1) to (8):

(1) X1-Leu-Asp-Leu-X2-Leu-X3 (where X1 denotes a set of 0 to 10 amino acid residues, X2 denotes Asn or Glu, and X3 denotes a set of at least 6 amino acid residues);

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3 (where Y1 denotes a set of 0 to 10 amino acid residues, Y2 denotes Phe or Ile, and Y3 denotes a set of at least 6 amino acid residues);

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3 (where Z1 denotes Leu, Asp-Leu, or Leu-Asp-Leu, Z2 denotes Glu, Gln, or Asp, and Z3 denotes a set of 0 to 10 amino acid residues);

(4) Asp-Leu-Z4-Leu-Arg-Leu (where Z4 denotes Glu, Gln, or Asp);

(5) α 1-Leu- β 1-Leu- γ 1-Leu;

(6) α 1-Leu- β 1-Leu- γ 2-Leu;

(7) α 1-Leu- β 2-Leu-Arg-Leu; and

(8) α 2-Leu- β 1-Leu-Arg-Leu

(where α 1 denotes Asp, Asn, Glu, Gln, Thr, or Ser, α 2 denotes Asn, Glu, Gln, Thr, or Ser, β 1 denotes Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His, β 2 denotes Asn, Arg, Thr, Ser, or His, γ 1 denotes Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp, and γ 2 denotes Gln, Asn, Thr, Ser, His, Lys, or Asp in formulae (5) to (8)).

5. The plant according to any one of claims 1 to 4, wherein material productivity per plant is significantly improved or reduced.

6. The plant according to claim 5, wherein material productivity per plant means ability to produce a protein in seeds.

7. A chimeric protein, which is obtained by fusing a transcription factor consisting of any one of the following proteins (a) to (c) and a functional peptide capable of converting an arbitrary transcription factor into a transcriptional repressor:

(a) a protein comprising an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76;

(b) a protein having transactivation activity and comprising an amino acid sequence that has a deletion, a substitution, an addition, or an insertion of one or a plurality of amino acids with respect to an amino acid sequence shown in any of the even-numbered SEQ ID NOS: 1 to 76; and

(c) a protein having transactivation activity encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide consisting of a nucleotide sequence complementary to a nucleotide sequence shown in any of the odd-numbered SEQ ID NOS: 1 to 76.

8. The chimeric protein according to claim 7, wherein the functional peptide has an amino acid sequence expressed by any one of the following formulae (1) to (8):

(1) X1-Leu-Asp-Leu-X2-Leu-X3 (where X1 denotes a set of 0 to 10 amino acid residues, X2 denotes Asn or

Glu, and X3 denotes a set of at least 6 amino acid residues);

(2) Y1-Phe-Asp-Leu-Asn-Y2-Y3 (where Y1 denotes a set of 0 to 10 amino acid residues, Y2 denotes Phe or Ile, and Y3 denotes a set of at least 6 amino acid residues);

(3) Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3 (where Z1 denotes Leu, Asp-Leu, or Leu-Asp-Leu, Z2 denotes Glu, Gln, or Asp, and Z3 denotes a set of 0 to 10 amino acid residues);

(4) Asp-Leu-Z4-Leu-Arg-Leu (where Z4 denotes Glu, Gln, or Asp);

(5) α 1-Leu- β 1-Leu- γ 1-Leu;

(6) α 1-Leu- β 1-Leu- γ 2-Leu;

(7) α 1-Leu- β 2-Leu-Arg-Leu; and

(8) α 2-Leu- β 1-Leu-Arg-Leu;

(where α 1 denotes Asp, Asn, Glu, Gln, Thr, or Ser, α 2 denotes Asn, Glu, Gln, Thr, or Ser, β 1 denotes Asp, Gln, Asn, Arg, Glu, Thr, Ser, or His, β 2 denotes Asn, Arg, Thr, Ser, or His, γ 1 denotes Arg, Gln, Asn, Thr, Ser, His, Lys, or Asp, and γ 2 denotes Gln, Asn, Thr, Ser, His, Lys, or Asp in formulae (5) to (8)).

9. A gene encoding the chimeric protein according to claim 7 or 8.

10. An expression vector containing the gene according to claim 9.

11. A transformant containing the gene according to claim 9.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2010/059495

A. CLASSIFICATION OF SUBJECT MATTER

C12N15/29(2006.01)i, A01H5/00(2006.01)i, C12N1/15(2006.01)i, C12N1/19(2006.01)i, C12N1/21(2006.01)i, C12N5/10(2006.01)i, C07K14/415(2006.01)n, C07K19/00(2006.01)n

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N15/00-15/90, A01H5/00, C12N1/00-1/38, C12N5/00-5/28, C07K14/415, C07K19/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA/BIOSIS/MEDLINE/SCISEARCH/WPIDS (STN), JSTPlus/JMEDPlus (JDreamII), GenBank/EMBL/DDBJ/GeneSeq, UniProt/GeneSeq

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/041693 A1 (Japan Science and Technology Agency et al.), 10 April 2008 (10.04.2008), table 1 (No. 1359); examples; claims & JP 2009-296886 A	1-11
X	WO 2003/013227 A2 (MENDEL BIOTECHNOLOGY, INC.), 20 February 2003 (20.02.2003), table 4 (G2533); table 6; pages 15 to 16 & US 2003/0226173 A1 & EP 1485490 A2	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
06 August, 2010 (06.08.10)

Date of mailing of the international search report
17 August, 2010 (17.08.10)

Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2010/059495

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The inventions according to items (a) to (c) in claim 1 relate to a plant in which a chimeric protein prepared by fusing a transcriptional factor to a functional peptide capable of converting a transcriptional factor to a translation inhibitory factor is expressed, and the inventions according to items (d) to (f) in the claim relate to a plant into which a gene encoding a transcriptional factor is introduced or in which an endogenous expression regulatory region for the gene is modified.

Only a common matter between these groups of inventions is a transgenic plant in which the function of a translational factor is modified.

(continued to extra sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Parts of 1-11 which correspond to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:77 and SEQ ID NO:78. (The search on the parts relating to SEQ ID NO:77 and SEQ ID NO:78 was substantially completed through the prior art search with regard to the unity of invention.)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2010/059495

Continuation of Box No.III of continuation of first sheet(2)

Document 1 (WO 2008/041693 A1) describes a transgenic plant in which a chimeric protein prepared by fusing a transcriptional factor to a functional peptide capable of converting a transcriptional factor to a translation inhibitory factor is expressed. Document 2 (WO 2003/013227 A2) describes a transgenic plant in which a transcriptional factor is overexpressed. It is considered that both Documents 1 and 2 describe a transgenic plant in which the function of a transcriptional factor is modified.

Thus, the former group of inventions and the latter group of inventions in claim 1 share no "special technical feature which makes any contribution over the prior art". Namely, the former group of inventions and the latter group of inventions do not comply with the requirement of unity of invention.

Next, the requirement of unity of invention is examined in the former group of inventions.

In items (a) to (c) in claim 1, 38 inventions which respectively relate to 38 sequences corresponding to the even-numbered sequences or the odd-numbered sequences among SEQ ID NO:1 to SEQ ID NO:76 are described in an alternative manner.

Although all of the 38 sequences are sequences for transcriptional factors derived from Arabidopsis, none of the 38 sequences is a sequence for a novel transcriptional factor. Further, there is no novel common sequence characteristic among the sequences.

In Document 1, a plant in which a chimeric protein prepared by fusing a transcriptional factor At2g23760 to a functional peptide capable of converting a transcriptional factor to a transcription inhibitory factor is expressed is described (see the section "examples", table 1, No. 462). At2g23760 is a transcriptional factor comprising the amino acid sequence depicted in SEQ ID NO:2 in the present application. Therefore, the plant described in Document 1 corresponds to the plant of the invention according to claim 1 of the present application.

Therefore, the provision of the plants of the inventions according to items (a) to (c) of claim 1 has no "special technical feature which makes any contribution over the prior art". Namely, the inventions according to items (a) to (c) of claim 1 and relating to plants into which chimeric proteins having different sequences are respectively introduced do not comply with the requirement of unity of invention.

Consequently, it is considered that items (a) to (c) of claim 1 include different 38 inventions respectively corresponding to 38 sequences.

Next, the requirement of unity of invention is examined in the latter group of inventions in claim 1.

In items (d) to (f) in claim 1, 4 inventions which respectively relate to 4 sequences corresponding to the even-numbered sequences or the odd-numbered sequences among SEQ ID NO:77 to SEQ ID NO:84 are described in an alternative manner.

Although all of the 4 sequences are sequences for transcriptional factors derived from Arabidopsis, none of the 4 sequences is a sequence for a novel transcriptional factor. Further, there is no novel common sequence characteristic among the sequences.

In Document 2, a transgenic plant in which a transcriptional factor G2533 is overexpressed is described (see table 4, table 6). G2533 is a transcriptional factor comprising the amino acid sequence depicted in SEQ ID NO:78 in the present application. Therefore, the plant described in Document 2 corresponds to the plant of the invention according to claim 1 of the present application.

(continued to next sheet)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2010/059495

Continuation of Box No.III of continuation of first sheet (2)

Therefore, the provision of the plants of the inventions according to items (d) to (f) of claim 1 has no "special technical feature which makes any contribution over the prior art". Namely, the inventions according to items (d) to (f) of claim 1 and relating to plants in which the sequences of the proteins of which the expression is to be modified are different from one another do not comply with the requirement of unity of invention.

Consequently, it is considered that items (d) to (f) of claim 1 include different 4 inventions respectively corresponding to 4 sequences.

As stated above, it is considered that claim 1 includes different 42 inventions which do not comply with the requirement of unity of invention.

Document 2 describes that a transgenic plant has an altered seed protein content (see column G2533 in table 2). Further, the phenotype of an altered seed protein content is inherently imparted to the plants described in Document 1 (see No. 462 in table 1). Therefore, a matter that the phenotype is imparted cannot be regarded as a special technical feature in the present application.

REFERENCES CITED IN THE DESCRIPTION

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